OUTLINES OF BACTERIOLOGY

DAVID ELLIS

With Author's Compliments
A W Bartlett

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(TECHNICAL AND AGRICULTURAL)

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BY

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PREFACE

THIS book is intended to serve as an introduction to Bacteriology in all its branches, though more attention has been bestowed on that aspect of the subject which is of most interest to students of technical and agricultural bacteriology.

Of late years, great strides have been made in this fascinating subject, and an attempt has been made to incorporate in the text the recent developments that are of fundamental importance. In some instances, as for example in our conceptions of the anaerobic bacteria, important changes have taken place. In other instances, however, our knowledge is still incomplete and rudimentary. Controversial questions, and matters requiring a technical knowledge of a special nature have been either omitted or have received only a passing reference. In all cases, where a technical application of bacteriology is discussed, the aim has been the demonstration of the fundamental principles which underlie that application, rather than the discussion of the details.

It has been deemed advisable to introduce a comparatively large amount of matter of purely theoretical interest, because in no other branch of science is a theoretical knowledge more necessary than in the science of Bacteriology. When one bears in mind that the organisms which are here discussed are visible only with the highest magnifications of the microscope, are very changeable in their nature, and are everywhere present around us, the need for a thorough theoretical training becomes obvious, even for those who wish only to make practical applications.

In addition to the published researches which have been consulted, I desire to acknowledge the help which I have obtained from the following books: Lafar's *Handbuch der technischen Mykologie*, Newman's *Bacteriology and the Public Health*, Green's *Fermentation*, and Muir and Ritchie's *Manual of Bacteriology*.

Further, I wish to acknowledge my indebtedness to the Rev. Robert Barr, M.A., to Prof. L. A. L. King, M.A., and to other friends for their valuable assistance during the compilation of this work.

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§ 1. INTRODUCTION.

It is generally acknowledged that Leeuwenhoek was the first to observe bacteria under the microscope, and his discovery of them was made in the year 1675. In order to test the power of his lenses he extracted from the human mouth what he regarded as promising material, and subjected it to observation. In this material he found small organisms which exhibited movement, and on this account, he called them *animalculae*, the idea at that time being that power of movement was confined to the animal kingdom. In 1683 his results, accompanied by drawings, were communicated to the Royal Society, which was then in its infancy. The forms which he described then were the cylindrical, spiral, and round kinds, which to-day are known, respectively, as *Bacillus*, *Spirillum*, and *Coccus*. But the importance of his discovery was not appreciated, even by Leeuwenhoek himself, and we have to wait for more than a hundred years before we see any further notice taken of these forms; then in 1786 appeared Müller's *Animalcula infusoria, fluviatilia et terrestria*. In this work also the bacteria are placed in the animal kingdom, though the author admits that they bear resemblances to some members of the vegetable kingdom. It is evident, in perusing Müller's researches, that the names he uses refer to *classes* of organisms, rather than to *species*.

The next work of note is that published by Ehrenberg in 1838. Although he still classified bacteria as organisms belonging to the animal kingdom, his work has the distinctive merit of marking off sharply the various genera.

His publication concerns itself with an examination of the Infusoria, which he divides into twenty-two families. Under one of these

families, viz. Vibriona, the bacteria are collected together into five genera:

(1) Bacterium. Cylindrical cells (Fig. 1*a*).
(2) Vibrio. Curved cells (Fig. 1*b*).
(3) Spirochaete. } Corkscrew shaped cells (Fig. 1*c*).
(4) Spirillum. }
(5) Spirodiscus. A variety of spirillum not since observed.

From this time onward the study of bacteria became a science in itself. As soon as Koch's work on Bacillus anthracis (1876), showed

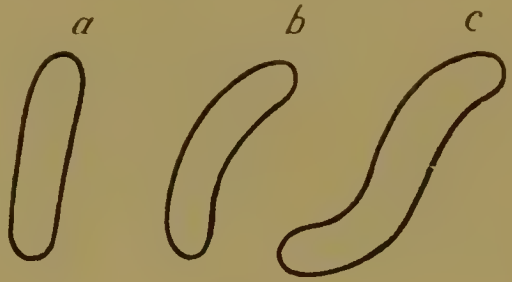

Fig. 1.

definitely the connection between bacteria and disease, and when, in the realm of technical industries, the important rôle of these small organisms was discovered, universal interest was awakened in them, and the results of investigators were eagerly awaited. At the present day this young science has developed to such an extent, that it has already many branches. The sum total of the gain to humanity obtained through the study of these minute forms, is considerable, and there are very few branches of other sciences in which a slight knowledge of the fundamental principles of Bacteriology is not useful.

CHAPTER I.

§ 2. FORMS OF BACTERIA.

The different forms which bacteria are known to assume may all be reduced to three types:

(1) Coccus, or round bacteria (Fig. 2*a*). All included under **Coccaceae.**
(2) Bacillus, or rod bacteria (Fig. 2*b*). ,, ,, **Bacteriaceae.**
(3) Spirillum, or spiral bacteria (Fig. 2*c*). ,, ,, **Spirillaceae.**

These shapes are constant; that is, a coccus-species always retains its spherical form, and the same can be said of the bacillus and spirillum. In the case of the bacillus group, however, it often happens that the rods are so small that they appear almost like cocci. This follows from the fact that any particular species has not always the same length, or even the same breadth, but if a species of this kind

Fig. 2.

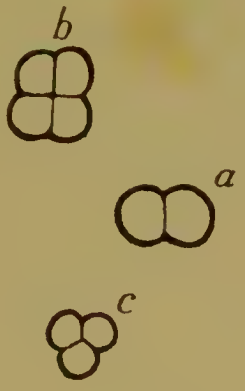

Fig. 3.

be constantly kept under observation, and cultivated in different ways, it will be found that the rod shape is constant, however much it may vary in length and breadth.

Looking more closely into the first group we find that the cocci arrange themselves in different ways. When division takes place, the

new cells that are formed do not always separate, but usually remain attached. When two cocci are bound together the combination is called a *Diplococcus* (Fig. 3*a*), if they are four in number and arranged in a square, we call them a *Tetracoccus* (Fig. 3*b*). More rarely may be found a plate of three cocci arranged in the form of a triangle. This is called a *Tricoccus* (Fig. 3*c*). Sometimes division takes place in all three directions of space, so that there is formed a mass of cocci clinging together.

Fig. 4.

The term *Packet Form* is applied to this mass, if the individuals are regularly arranged (Fig. 4*a*). If, however, the cocci are irregularly arranged, resembling, for example, a bunch of grapes, the term *Staphylococcus* is applied to the group (Fig. 4*b*).

Finally the division may take place in one direction only, so that the cocci arrange themselves to form a chain. This is called the *Chain Form* (Fig. 4*d*). These various methods of combination enable us to subdivide the cocci-species of bacteria into classes, because it has been found that some species divide, for example, to form chains, and never in any other way. A classification of the cocci forms, based on these facts, has therefore been drawn up as follows:

I. **Streptococcus.** Those which divide to form a chain of cocci (Fig. 4*d*).

II. **Micrococcus.** } The species belonging to these two groups divide
III. **Planococcus.** } always in the same two planes, so that a plate of cocci is formed. They may form diplococci, tricocci or tetracocci, or even groups of more than four (Fig. 4*c*), but they never divide in three directions of space. The name Micrococcus has been applied to each of the species of this description which is non-motile, whereas the name Planococcus is given to each of the motile species.

IV. **Sarcina.** } These two groups differ from II. and III. in
V. **Planosarcina.** } that, in addition to showing the methods of combining together followed by the latter, they are also able to divide in all three directions of space, *i.e.* they have the power of forming

packets (Fig. 4*a*) or Staphylococci (Fig. 4*b*). If these species are non-motile they belong to the *Sarcina* group, but if motile they belong to the *Planosarcina* group.[1]

Turning now to the bacillus, or rod-shaped individuals, we find that they also show great variety in form, and in the ways in which they

Fig. 5.

Fig. 6.

combine together. It has already been said that there is no constancy in the length or breadth of the individuals of any one species. A species which, cultivated in one medium, exhibits short thick rods, may develop into long thin threads in another medium. When cultivated in wort, Bacillus Kützingianum shows very short, thick individuals as seen in Fig. 5, but when cultivated in a solution containing flesh extract and peptone, long threads are developed similar to those shown in Fig. 6.

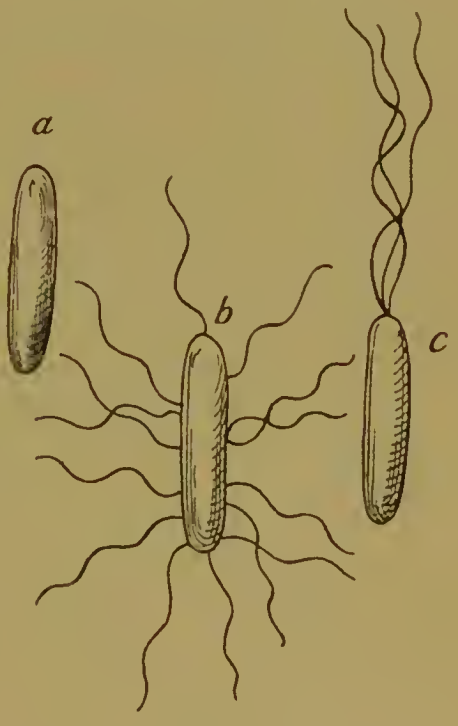

Fig. 7.

In sub-dividing the rod-shaped species, advantage is taken of the fact that some are not motile, whilst others possess motility; and, further, that those that are motile show differences in the mode of insertion of the organs of motion. These organs of motion, sometimes called *cilia*, and sometimes *flagellae* (singular *cilium* and *flagellum*) are whip-like filaments of living matter thrust out from the sides of the organisms, and it is the lashing of the cilia that causes the movement of the organism to which they are

[1] The author has lately shown the probability that the distinction between Micrococcus and Planococcus, and between Sarcina and Planosarcina, is not a real one, for all the species belonging to the immotile Micrococcus and Sarcina

attached. In accordance with these facts the rod-shaped bacteria are classified as follows:

I. **Bacterium.** Immotile (Fig. 7*a*).

II. **Bacillus.** Motile, the cilia being arranged all round the organism (Fig. 7*b*).

III. **Pseudomonas.** Motile, the cilia being found only at the poles (Fig. 7*c*).

These three genera include all the rod-shaped bacteria, the individuals of which are normally quite free. Sometimes under certain conditions the rods form in rows, end to end, like a string of sausages. This is a characteristic mode of growth of Bac. anthracis (Fig. 8).

The third fundamental type is the *Spirillum* or spiral form. In the first place, each individual of a species may assume the form of a

Fig. 8.

Fig. 9.—Vibrio form.

"bent" rod. This is known as the *Vibrio* form of growth (Fig. 9). It is exhibited in the dreaded organism which causes cholera. This is not strictly a spiral, but rather an intermediate form between the true rod shape and the true spiral, and is best included here.

It will readily be seen that those bacteria, which exhibit twists, will show a variety of different forms, according to the number of the twists, and the closeness or looseness of the spirals. The classification of these forms is based, like that of the rod-bacteria, on the presence or absence of cilia.

We have

I. **Spirosoma.** Spiral cells without organs of motion (Fig. 10).

II. **Microspira.** Spiral cells with 2–3 cilia at the poles (Fig. 11).

III. **Spirillum.** Spiral organisms with 5–20 polar cilia (Fig. 12).

groups, examined by him, could, by appropriate cultivation be made motile. As the supposed non-motility of Micrococcus and Sarcina is the only characteristic separating them from Planococcus and Planosarcina respectively, it is extremely probable that the five groups mentioned above must be reduced to three, viz. Streptococcus, Planococcus (or Micrococcus), and Planosarcina (or Sarcina). The author has also found that all the Streptococcus species examined by him could be rendered motile by appropriate cultivation (*Centralblatt für Bakteriologie*, Abth. ii. Bd. ix.).

IV. **Spirochaete.** Spiral organisms which move by undulations of their body and not by means of cilia (Fig. 13). Each Spirochaete organism consists usually of a large number of twists.[1]

Fig. 10.—Spirosoma.

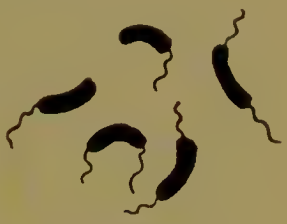

Fig. 11.—Microspira.

Fig. 12.—Spirillum, showing cilia.

This concludes the list of what may be designated as Bacteria in the narrower sense of the term. There are, however, a number of other organisms which are placed among the bacteria. These represent a higher form of development, because they show a more complex type of structure and in many cases a banding together of individuals to form a community. These organisms are usually regarded as forming the group **Chlamydobacteriaceae.** We shall mention here a few of the more common types in order to show the variety of forms which they can assume, leaving to later sections a fuller description of their life-histories and physiological characteristics.

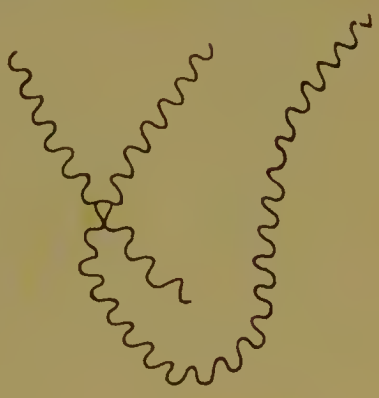

Fig. 13.—Spirochaete.

[1] It is extremely doubtful whether the members of the genus Bacterium are always motionless. In all the forms examined by the author it has been found possible to induce motility by cultivating them under conditions in which they were in contact with their own excretion-products as little as possible. If such be the case, the distinction between *Bacterium* and *Bacillus* is done away with (*Centralblatt für Bakteriologie*, Abth. ii., 1903).

1. **Streptothrix.** Simple undivided threads. Division takes place in one direction of space only (Fig. 14).

2. **Cladothrix.** In this genus the threads are composed of small rods enclosed in a membranous tube (Fig. 15). The appearance presented

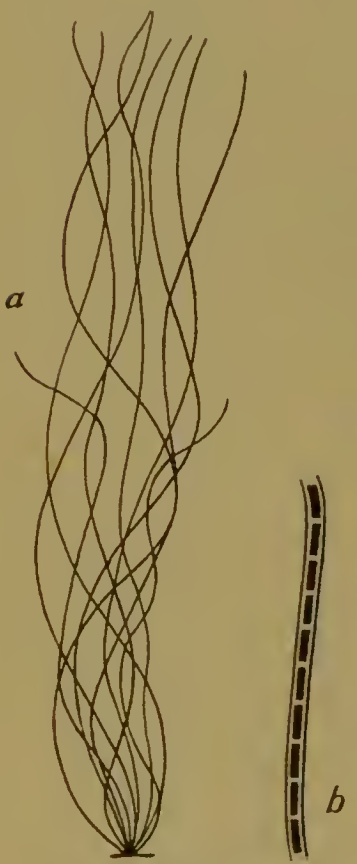

Fig. 14.—Streptothrix. (*a*) Under low power of microscope; (*b*) small portion very highly magnified. (After Migula.)

Fig. 15.—Cladothrix. (After Zopf.)

Fig. 16.—Cladothrix dichotoma, showing false branching. (After Zopf.)

by a colony of Cladothrix dichotoma is shown in Fig. 16. There is no true branching, as the apparent branches are formed by the slipping aside of single cells, which subsequently form threads, but remain attached to the parent plant. Here we see a good example of a community of individuals. Each thread is made up of a number of short rods (similar to those of the genus bacillus) and the membrane

which encloses the rods has been supplied by means of a contribution from each of the rods constituting the thread.

Fig. 17.—Crenothrix polyspora. (After Zopf.)

3. **Crenothrix**. The threads constituting this genus are similar to those of Cladothrix, but differ in that multiplication of the enclosed rods takes place in all directions, so that a cluster and not a row of cells is formed inside the membrane, as represented in Fig. 17.

4. **Phragmidiothrix**. The appearance of this complicated form is sufficiently indicated by Fig. 18.

5. **Leptothrix.** Threads with very sharply defined stiff membranes (Fig. 19).

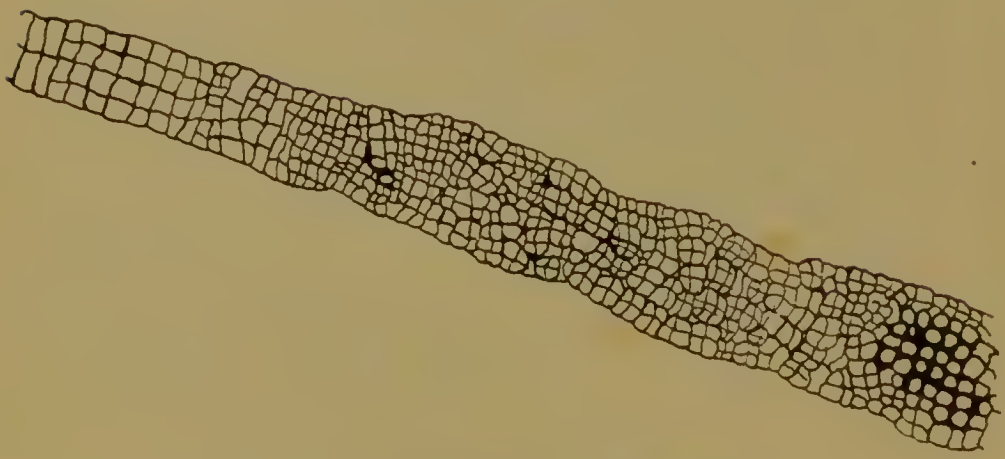

FIG. 18.—Phragmidiothrix. (After Migula.)

6. **Gallionella.** In this genus the thread has the appearance of a hairpin twisted on itself (Fig. 20).

FIG. 19.—Leptothrix.

FIG. 20.—Gallionella.

FIG. 21.—Spirophyllum.

7. **Spirophyllum.** The organism consists of a spirally twisted flat band (Fig. 21).

§3. THE SIZE AND DISTRIBUTION OF BACTERIA.

Bacteria are the smallest of known organisms, the breadth of an average bacillus being about one μ (μ is the unit of length in Bacteriology and $= \frac{1}{1000}$ millimetre; a millimetre = approx. $\frac{1}{25}$ inch). The average length of the rod-bacteria may be taken as three or four times the breadth, though some may be found much smaller. The very smallest that we know of, have a breadth of $\frac{1}{10}\mu$. With regard to the length, although the average is that given above, long threads are by no means uncommon, this result being found frequently when the conditions of growth are so unfavourable that the bacteria are unable to divide. Some of the Chlamydobacteriaceae, however, may normally attain to a length of as much as 200 μ, as, for example, Leptothrix ochracea. This is $\frac{1}{5}$ mm., an enormous size for a member of this class of organisms. As the length of an individual depends on the activity of cell-division, and as this is not the case with regard to the breadth, it follows that variation in length is much more common than variation in breadth. With regard to the Coccus-group also, 1 μ may be taken as the average diameter of an individual. Many species, however, have smaller diameters, whilst a few attain even double this diameter, and all sizes between these two extremes are found. The same variation is found in the diameter of the individuals that compose any one of the round variety of bacteria, and in the length, breadth and number of twists of the spiral forms. The largest known spirillum is Spirillum giganteum, in which the diameter of its spirally wound thread measures from $1\frac{1}{2}\mu$ to 2 μ.

An idea as to the size of bacteria may be obtained by imagining a number of bacilli of average dimensions set up in a row as represented in Fig. 22. It would take about 2000 of these to stretch across the head of a small pin, whilst about 20,000 of the smallest bacteria would be required to cover it.

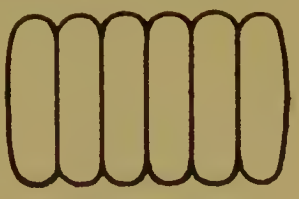

Fig. 22.
(For explanation see text.)

With regard to the distribution of these organisms, we find them present in all except in a very few places. These exceptions are necessarily places where owing to unfavourable circumstances, such as an inadequate amount of moisture or of food, or a very low temperature, no vegetative life of any kind is possible. Thus the tops of high mountains, arid deserts and the

regions round the North and South Poles are free from bacteria. The manner of their distribution will be more fully dealt with in a later chapter. It will suffice here to state that it is very uneven, for whilst some substances like sewage and milk contain several millions of bacteria per gram, others like sand contain normally a very small number.

§4. DO BACTERIA BELONG TO THE VEGETABLE OR TO THE ANIMAL KINGDOM?

The most common mistake that is made concerning bacteria is that they are all minute animals. This is a mistake, due, probably, to the fact that people find it difficult to associate with the plant life they are familiar with, organisms which are free and motile, characteristics commonly supposed to be monopolies of the animal kingdom. At first even scientists classified bacteria as belonging to the animal kingdom. Although we have no difficulty in allocating a cabbage to the vegetable kingdom, or a dog to the animal kingdom, when we come to examine organisms much lower down in the scale of life we find that the characteristics of the animals and the plants tend to approach each other. In fact, there are several organisms of a low scale of organisation that are claimed both by zoologists and by botanists as belonging to their respective kingdoms. Such for instance is Volvox, a description of which will be found in text-books both of zoology and of botany. In the case of such a doubtful form, the judgment is made on the sum total of its characteristics. With regard to bacteria, with the possible exception of the spiral forms, their characteristics proclaim them to be members of the vegetable kingdom. Their methods of reproduction, of cultivation and of cell-division are so distinctly of the nature of plants, that since the life-history of these bacteria has been accurately known, there has not been any doubt in the minds of scientists as to their insertion inside the vegetable kingdom. There is some doubt, however, as to the inclusion of the spiral bacteria within the vegetable kingdom; this applies most particularly to Spirochaete, the characteristics of which approximate very closely to organisms that indubitably belong to the animal kingdom.

§5. THE INTERNAL STRUCTURE AND CONTENTS OF THE BACTERIAL CELL.

Although these organisms are very minute, some headway has been made in our knowledge of the structure and contents of the bacterial cell. Like all other organisms, bacteria possess within their cells that substance which is the seat of life, viz. *protoplasm*. This protoplasm in bacteria is usually enclosed by a protective membrane or cell-wall, which is secreted by the protoplasm. The cell-wall bears the same relation to the protoplasm that the nails of our fingers bear to the living substance of our fingers.

We do not know the structure of protoplasm, for it cannot be analysed without being killed, and when it is killed the structure is altogether changed. This applies to the structure of protoplasm generally, and not merely to that of bacteria. We know, however, the chemical elements that enter into its composition. These are carbon, oxygen, hydrogen, nitrogen, sulphur, and phosphorus. Now, in all living creatures, protoplasm is being constantly built up, and again broken down into simpler substances. Its production consequently necessitates a constant supply of *raw materials* of different kinds. These constitute the food of an organism. As the raw material is not changed into protoplasm at one step, there will be a number of substances inside the cell, intermediate between raw material and protoplasm. These we may term *intermediate products*. Then again, when the protoplasm breaks down, another set of different substances is formed. There will, therefore, be a large number of substances inside the cell, as a result of these two processes. Then it must be remembered that many of the substances absorbed as raw material cannot be used as food in the form in which they are absorbed, and therefore they are changed into the appropriate form by substances called *ferments*, which will be treated more fully in a later chapter.

Further, protoplasm is sometimes specialised, portions of it being set apart to perform certain functions. Thus, in higher plants, a specialised part, called the *nucleus*, is mainly concerned with the reproduction and multiplication of cells, and as another example may be mentioned the *chlorophyll corpuscles*, which cause the colour of green plants and are concerned in the elaboration of plant-food. We shall now proceed to what has actually been discovered in the case of bacteria.

1. **Protoplasm.** As in all other organisms, so in bacteria this is a semi-liquid, semi-solid substance. It can be stained with the usual dyes, the best in the case of bacterial protoplasm being solutions of methylene-blue, iodine, Bismarck-brown, or fuchsin. In higher plants increase of the volume of the cell is more than the protoplasm can keep up with, and consequently spaces, called *vacuoles*, are left in the cell, which are not filled with protoplasm. The vacuoles are filled with *cell-sap*, which consists usually of a mixture of raw materials, designed for the building up of fresh protoplasm, and other materials produced by the breaking down of protoplasm. Vacuoles have been demonstrated in bacterial cells (Fig. 23), and we may reasonably assume that they are filled with substances of the same class as the vacuoles of the higher plants, though this cannot be demonstrated. The protoplasm can be made to contract from the cell-membrane which encloses it

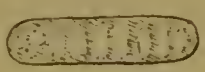

FIG. 23.—Cell showing vacuoles.

FIG. 24.—Plasmolysed cells. Shows clear space between wall and protoplasm.

by placing the organism in a substance like common salt, which abstracts a portion of the water from the protoplasm (more than 75 per cent. of protoplasm consists of water). This causes it to contract, thus separating it from the membrane (Fig. 24). A cell thus treated is said to be *plasmolysed*.

With regard to the specialised portions of protoplasm, the first we have to deal with is the nucleus. Many and various are the opinions which are held with regard to the presence or absence of this organ, but we need not enter into the numerous views entertained on this subject. It will be sufficient to mention that by appropriate staining it is possible to demonstrate small round substances always enclosed in protoplasm, which, in number, relative size, and staining capacity, correspond to similar structures which are found in the cells of fungi. It is probable that these are nuclei, but since other small round substances are often found in the cell, which we know are not nuclei, and which stain in a similar fashion, there is a certain amount of uncertainty, the more so because these are too small to admit of their

finer structure being demonstrated. The whole question must be regarded as still an open one.

Next, with regard to portions of protoplasm specialised for purposes of food-production, the only case that we know of is that of a section of the sulphur-bacteria, the members of which are coloured purple. The colouring matter—called bacterio-purpurin—is stated to perform the same functions for these bacteria as chlorophyll does for green plants, that is, it is the means by virtue of the possession of which these bacteria, unlike all others, are able to absorb carbon dioxide, and convert it into a more complex compound which can be used as food by them.

There are many other coloured bacteria, but the colouring matter in these has either been excreted and is found outside the cell, or appears inside the cell as a secretion of the protoplasm. It is not a living substance, nor is it a part of protoplasm specialised to perform a definite function. The cilia should also be mentioned here as being specialised portions of protoplasm. These structures will be fully dealt with in later sections.

2. **Raw Materials, Intermediate Products, and Secretion Products.** These must be treated together, because often we cannot tell whether any particular substance found in the cell is a raw material, or has been formed by the breaking down of protoplasm. In most bacteria no indication of the presence of these materials is given by treatment with stains, but in a few some of these have been demonstrated, which are chiefly in the form of *reserve material*. When the supply of raw material or any particular form of intermediate products is in excess of the demand, the surplus is put aside and is called a reserve material. Thus, in the higher plants, when the supply of sugar is greater than is immediately required, the excess is stored in the form of starch, which is changed back into sugar when sugar is again wanted.

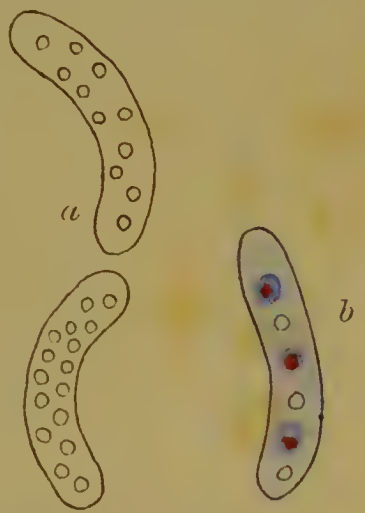

FIG. 25.—Spirillum gigantenm. (*a*) Unstained; (*b*) stained with methylene-blue. Volutin—blue with purple centre. Fat unstained.

A number of these reserve materials have been demonstrated in bacteria, although it is not known exactly how they have arisen. Thus in Spirillum giganteum, the cell in healthy cultures is found to be full of *fat-globules*, and another reserve material called *volutin* (Fig. 25*a*). If a

healthy culture of Spirillum gigantenm be examined microscopically when one day old, the cells will be found quite full of these reserve materials, but if the culture be examined after five or six days, it will be seen that the amount has very considerably diminished, because the spirillum had need of the reserve and consequently used it. The fat-globules can be identified by their strongly refractive appearance, and by their reactions, and can be further distinguished from the volutin-spheres by the fact that methylene-blue stains the latter but not the former. Volutin has also this further peculiarity, that when stained with this reagent the central portion of the larger spheres is purple, and the periphery blue in colour (Fig. 25*b*). Fat-globules and volutin

Fig. 26.—Bac. tumescens, showing fat globules.

have also been found in several other species of bacteria, *e.g.* fat-globules are almost always found in healthy cultures of Bac. tumescens (Fig. 26) and Bac. tuberculosis, whilst volutin has been found in Bac. alvei and in other bacteria.

Another reserve material is the carbohydrate, *glycogen*, which, in Bac. asterosporus, makes its appearance shortly before the formation of spores. This substance is recognised on treatment of the cells with iodine, when irregular bodies of a very deep reddish-brown colour will be observed, staining much more intensely than the surrounding protoplasm (Fig. 27).

Fig. 27.—Bac. asterosporus, showing grains of glycogen. Stained with iodine.

Another reserve material has been found in some of those bacteria which excrete butyric acid. This material appears just before the formation of spores. It is probably allied to starch, for when treated with iodine it, like starch, stains blue.

Finally, we take the case of those bacteria that are coloured. In some cases, as has been stated, the colour lies inside the cells, the colouring matter being a product resulting from the breaking down of protoplasm, which colouring matter must therefore be included in a list of substances that can be identified inside the cell. This concludes the list of substances that may be identified by the use of the microscope. By taking into account the results of chemical analyses of bacterial cultures, the

list may be considerably extended. In this way a large number of acids (*e.g.* butyric, lactic, acetic), esters, and alcohols, have been identified: also methane, carbon dioxide, hydrogen, sulphuretted hydrogen, ammonia compounds, nitrous- and nitric-compounds, free nitrogen, and many others. Some are extruded from the cell in the form of gas, others are extruded in the liquid or solid condition, whilst a third group is retained in the cell, chiefly in the vacuoles. A complete list of all the substances that are found inside the cells of bacteria would probably include a very large percentage of all the organic compounds known to chemists.

CHAPTER II.

§ 1. THE CILIA OF BACTERIA.

1. **General Nature.** The *cilia*, or *flagellae* as they are sometimes called, are whip-like filaments of protoplasm projecting from the sides of the cell, which, by their activity, cause the bacteria to move through the liquid in which they are placed. They are not visible

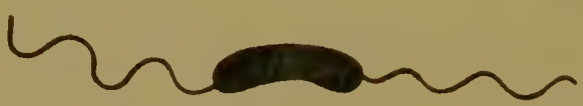

Fig. 28.—Microspira, monotrich ciliation.

when observed under the microscope unless they have been stained in a particular way; their form is almost always that shown in Fig. 7*b*, viz. wavy filaments, the number of waves depending on the length of the cilium and the nature of the species. Sometimes long cilia, looking like bent willow branches, are to be seen, but it is probable that these are not normal structures. Cilia are not all inserted alike; the following kinds may be distinguished:

(1) Monotrich. One polar cilium (Fig. 28).

(2) Peritrich. Cilia all round the cell (Fig. 29).

(3) Lophotrich. Groups of polar cilia (Fig. 30).

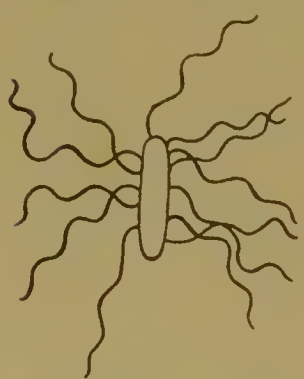

Fig. 29.—Bacillus showing cilia (peritrich ciliation).

Among bacteria the number of constant characteristics is very small, but among this number the mode of insertion of the cilia is to be placed. If an organism has polar ciliation, under all circumstances the ciliation remains polar. This is particularly useful for purposes of

classification. With the exception of Cladothrix dichotoma, all the polar cilia are found at the very end of the cell. In the organism mentioned, however, the cilia are a very little distance to the side,

Fig. 30.—Spirillum, showing lophotrich ciliation.

as is seen in Fig. 15. Cilia are to be found in the round, as well as in the rod and spiral organisms. Fig. 31 shows a preparation of Sarcina ureae, Fig. 32 one of Micrococcus citreus, and Fig. 33 one of Streptococcus pyogenes. We thus see that all the three great divisions of the cocci-bacteria possess ciliated species. It may be stated generally that all the spiral bacteria have polar, and all the cocci (with the exception of the genus Streptococcus) peritrich ciliation. The rod forms have representatives of both kinds. The cilia are always of uniform thickness for the same species, but are undoubtedly thicker

Fig. 31.—Sarcina ureae, showing mode of ciliation characteristic of this genus.

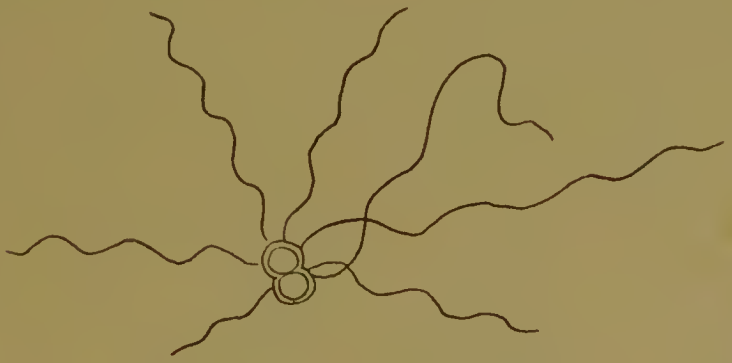

Fig. 32.—Micrococcus citreus, showing cilia.

and stronger in some than in others. They are never branched, and consist of thin colourless filaments of protoplasm.

Development of Cilia. The development of cilia has been followed in the case of Spirillum giganteum (often erroneously called Spirillum volutans), in which they are polar and strongly developed. The

different stages in the development can be seen in Fig. 34. In this case it is obvious that all the polar cilia of one individual arise together,

FIG. 33.—Streptococcus pyogenes, showing cilia.

develop at the same rate, and attain their full development at the same time. It has been stated by several writers that the cilia arise, not

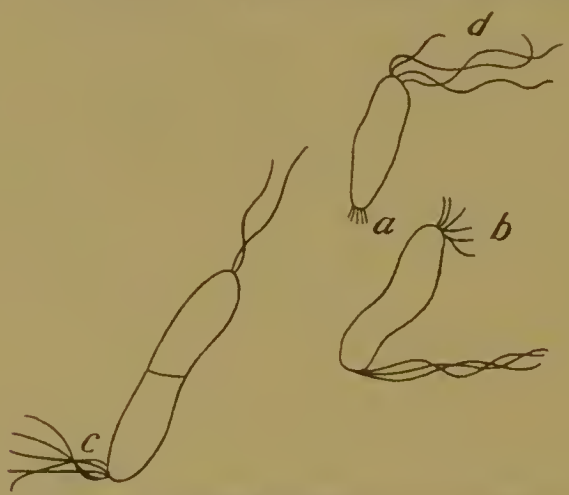

FIG. 34.—To illustrate the development of cilia. Various stages of development represented at *a*, *b*, *c*, and *d*.

from the interior, but rather direct from the membrane, the contention being that the membrane is not altogether a lifeless layer, but partakes somewhat of the nature of protoplasm itself. As, however, the course of the cilia has been followed *through* the membrane (Fig. 35), there can be no doubt that they cannot arise *from* the membrane.

FIG. 35.—Spirillum gigantcum, showing origin of cilia from interior of cell.

Nature of Motility. In virtue of the possession of cilia, bacteria are able to exhibit motion. Even in solid nutrient media there is usually quite enough water on the surface for the bacteria to swim in, even although to our eyes it appears quite dry. As seen in the various diagrams the cilia are wavy in outline, and it is by their lashing that the movement of the bacteria is caused. In the bacteria of the coccus kind, movement takes the form of rotation in any direc-

tion, or a violent trembling of the organism may be the only result of the activity of the cilia, or finally there may be a forward movement unaccompanied by either trembling or rotation. In the rod and spiral bacteria also, we see the same movements, except that the latter do not exhibit a rotatory movement, and in the former this kind of movement is very uncommon. The rotation of an individual belonging to the rod-bacteria is confined to an occasional turn round, without moving forward. When an obstacle is encountered the individuals seem to have no difficulty in changing their direction, and occasionally they may be seen retracing the path by which they have just come. In general the spiral varieties have more rapid movements than the others, and by appropriate cultivation it is possible to increase this rapidity. In the author's experiments with Spirillum giganteum, an increase of rapidity was obtained in the following way : A fresh culture of this species was made every day, the material for each inoculation being taken from the youngest culture, which would thus be 24 hours old. After a month or two of this treatment, the individuals of young cultures of this species were so motile that when examined microscopically in a drop of water the microscope-field presented a blurred appearance as if out of focus. The cilia preparations of such cultures showed about 30 or more cilia at the poles of some of the individuals. Some bacteria are so slow in their movements that it is often difficult to tell whether they are moving or not. If the movement is very slow it is difficult to distinguish it from the Brownian or molecular movement which the smallness of the bacteria subjects them to. An average speed is from 3 μ to 6 μ per second. The great differences that exist in the rate of movement is partly due to the variation in the number and strength of the cilia, and also partly to the variable nature of the mucilaginous layer which is found covering the cell-wall, by which it has been produced. The greater the amount of mucilage the slower the movement, and of course the converse also holds true.

Species normally motile often go through a portion or even the whole of their life-history without exhibiting motion of any kind.

Some bacteria are motile during the whole, whilst others are motile for only a small portion of their lives. In the case of those species which form spores, motility usually begins immediately after germination. Gottheil in examining the germination of a number of soil bacteria found that the time of the inception of movement varied for different species from 7-12 hours after "sowing" the spores. When once started, in the majority of cases, movement continues as long as

the bacteria are alive. What usually happens in a tube culture is that the excretion products of the bacteria ultimately effect their destruction, and naturally the motility is lost when the protoplasm is destroyed. In the case of those species which form spores, the formation of these structures involves the abstraction of protoplasm from the remainder of the cell. Consequently the cilia, being detached from their base, fall off, and movement ceases. It is by no means uncommon, however, to see motile individuals possessing spores. This results from the fact that all the protoplasm has not been used up in spore-formation, a notable example being the case of Sarcina ureae.

The cilia are very delicate structures, and unfavourable external circumstances often cause them to fall off, although the individuals are not dead. In other cases, without falling off, they may become paralysed, thus causing a cessation of motility. We know this because we can sometimes demonstrate the presence of cilia in organisms which are motionless, but which have been previously in active motion.

§ 2. CELL-DIVISION.

Multiplication among bacteria is effected almost entirely by division of cells, whereby two individuals are formed out of one. After division, the two daughter-cells separate, grow into mature cells, and very soon repeat the process of division, so that, in a comparatively short time, if conditions are favourable, many millions may be formed from a single individual. The process of division can easily be followed by comparing individuals in an actively growing culture, when, if the preparation has been properly stained, the various stages are exhibited.

1. **Division in the Bacteriaceae.** This can be followed by comparison of the individuals shown in Fig. 36. As seen in *b*, the first sign of division, after slight elongation, is the formation of a wall at right angles to the long axis of the cell. This wall is very thin at first, and does not show the double contour characteristic of the mature state. It usually divides the cell into two equal halves, though sometimes the parts are unequal. The next stage is exhibited in *c*, in which a constriction has taken place in the middle, at the point where the new wall is located. The subsequent stages are accomplished in two ways. In the first, shown in *d*, *e*, *f*, the constriction deepens (*d*), and this is followed by a splitting of the division-wall into two halves, separated by a thin clear line which does not take up the stain (*e*). This clear line is composed of a thin mucilaginous layer, formed by a change of

the cell-wall into mucilage at this part. The final stage (*f*) consists of a rounding off of the ends of the daughter-cells at the point of junction, after which they gradually draw apart, the substance connecting them is dissolved, and henceforth the two halves lead a separate existence. Each grows to maturity, and the process of division is repeated. In the other way, the process of cell-division is completed as shown in *d'*, *e'*, in which the thin clear line appears very soon after the beginning of the constriction (*d'*). The subsequent development is

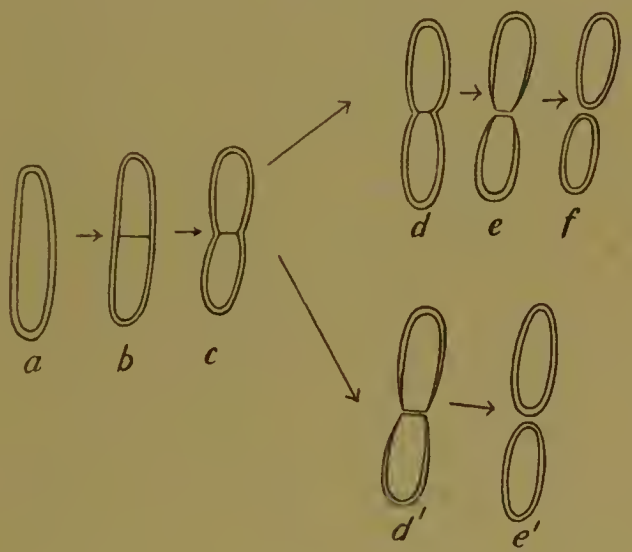

FIG. 36.—Cell-division in rod-cells. (For explanation see text.)

the same, viz. a rounding off and separation of the cells (*e'*). Whilst this latter process is going on the new cell-wall that has been formed at the line of separation of the two daughter-cells thickens, until the wall of the new cell is uniform throughout (*f* and *e'*). In some cases the mucilaginous substance which binds the two daughter-cells does not immediately dissolve, so that one sometimes sees two newly formed daughter-cells separated by a distance equal to their own length, but evidently still connected by an invisible thread of mucilage, which can, however, be made visible by appropriate staining.[1]

2. **Cell-division in the Coccaceae.** In healthy cultures the predominant forms are one one-, two-, three-, and four-celled individuals, which, unlike the bacillus group, usually divide before expansion takes

[1] Cell division in the genus Pseudomonas has not yet been investigated. There is very little doubt, however, that in all essentials, this process agrees with that observed in the genus Bacillus.

place. This is seen in Fig. 37*a*, in which each cell has two division-walls. In this particular case it is evident that each cell is destined to become a four-celled group (Tetracoccus). The further development of each of these is represented in Fig 37*b*, *c*, *d*, *e*; expansion and constriction take place till we have the stage represented in *b*, then the cell-group may thicken its new walls to form the stage shown by *c*, after which its development is complete. It may, however, proceed further by splitting up as represented in *d*, in which the four cells are separated by a thin mucilaginous cross. In *e* the separate cells round themselves off, complete the thickening of the walls, and then separate, becoming one-celled individuals (Unicocci). The formation of two-celled individuals (Diplococci) takes place by a further development of the *e* stage, in which only one of the inner cross-walls forms a separating mucilaginous line. The stages are shown in Fig. 37*f*, *g*. A three-celled individual (Tricoccus) is formed from the Diplococcus by the further division of only one of the cells.

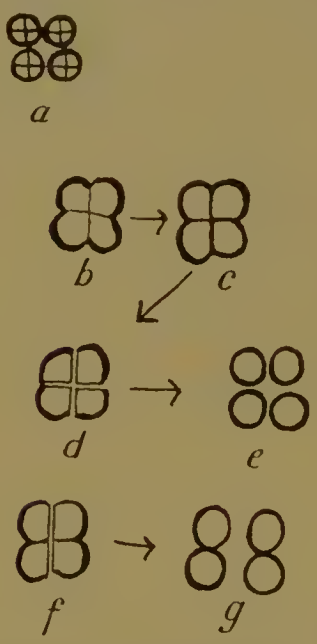

FIG. 37.—Cell-division in round-cells. (For explanation see text.)

In healthy cultures, the cell-groups very often consist almost entirely of mono-, diplo-, tri-, and tetracocci: under somewhat different conditions, the cocci do not separate as soon as formed, but remain attached, and undergo further division. The result of this is that packets of cocci, resembling bales of cotton (Fig. 38) are formed. Many of the cocci composing these packets have division-walls, the division in these having gone no further than the formation of walls. When some of the large bales are moving slowly, it can be seen that in many of them division-walls are found in three directions of space, that is to say, a coccus is divided into octants, and each wall is at right angles to the other two. This shows that division takes place in three directions of space.

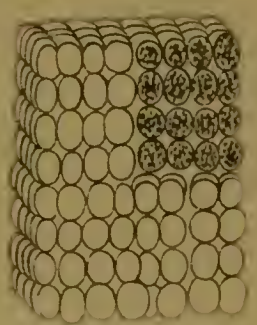

FIG. 38.—Cocci united to form packets.

With regard to the formation of micrococci, *i.e.* cocci which divide only in two directions of space, producing a *plate* of cells, it will be

readily seen that the division of these cells is exactly the same as for Sarcina except that division-walls are in two instead of in three planes. The process is similar when Streptococci are in process of formation. In this case chains of cells are produced.

3. **Cell-division in the Spirillaceae.** In this order the method of cell-division is essentially different from that followed by the Bacteriaceae and Coccaceae. It has been observed in Spirillum giganteum, which on account of its large size is admirably suited for the purpose. The normal undivided cell is represented in Fig. 39*a*, the

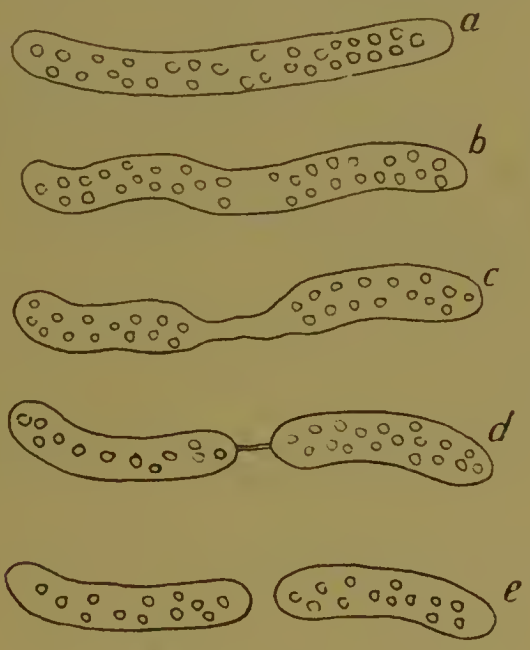

FIG. 39.—Mode of division in spiral cells.

globular contents being reserve materials composed of fat-globules, and volutin. In the first stage of cell-division, the reserve materials are seen to be wanting in a certain area in the middle, which area marks the spot where cell-division is about to take place.

When stained so that the wall is shown, it is found that in it, as yet, no change has taken place (Fig. 39*b*). In a slightly more advanced condition, the reserve materials are seen to have so arranged themselves that the separation-area is, roughly, like a broad, double, concave lens in appearance (Fig. 39*c*), and when this stage is examined after staining, the wall is found to be still unchanged (Fig. 39*d*). In the next, and following stages, constriction of the separation-area takes place, with the result that the connection between the two halves gets smaller and

smaller, until ultimately complete separation ensues. When stained specimens of these stages are examined, in no case is it found that a wall is thrown across as in the Bacteriaceae and Coccaceae. Instead, the walls on either side follow the constriction (Fig. 40), and ultimately meet. The walls of each daughter-cell, at the separation-area, are trimmed to conform to the shape of the cell, and the only connection between them is a thread of mucilage, which, however, soon disappears, leaving the two cells free.

Fig. 40.—Stages in division of spiral cells. (See text.)

The process in this genus is absolutely different in principle from any process of cell-division inside the vegetable kingdom. It is, in essentials, a division by fission, similar to the process among the lower organisms in the animal kingdom. It differs, however, in the possession by these organisms of a definite cell-membrane.

4. **Cell-division in the Thread-bacteria.** Cell-division in the organisms included under Chlamydobacteriaceae has not been accurately studied. We have seen that there are two types of cell-division, one in which the partition is initiated by the formation of a new cell-wall across the cell (Bacteriaceae and Coccaceae), and one in which it is initiated by a process of constriction (Spirillaceae). There can be little doubt that in the various members of the Thread-bacteria the method of cell-division follows one or other of these two types. In Gallionella ferruginea and Leptothrix ochracea, although we do not know all the details of cell-division, we known enough to be able to state that it belongs, in essentials, to the method which obtains in the Spirillaceae. We shall deal with these two species when we come to the treatment of the Iron-bacteria.

§ 3. SPORES.

Vegetative reproduction, as seen above, is effected by cell-division followed by growth of the daughter-cells, a method which enables the bacteria to multiply very rapidly. The capacity of resistance of

these cells, however, is very small. Many bacteria, therefore, interrupt their growth and multiplication by entering into a resting condition, in which no growth or multiplication takes place until favourable circumstances once more arise. Any portion of a bacterial cell which is separated from the rest for this purpose is called a *Spore*.[1] If this portion is formed inside the cell it is called an *endospore* (Fig. 41). The formation of endospores is very common in the Bacteriaceae, is found only in two species in the Coccaceae, and never in the Spirillaceae, nor in any of the higher bacteria (Chlamydobacteriaceae) so far as is at present known.

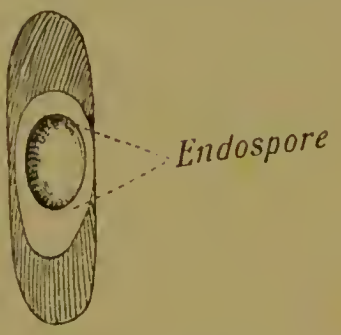

Fig. 41.—Bacillus in which an endospore has formed.

The endospores are thick-walled glistening structures of an oval or round shape. Each contains dense protoplasm and reserve materials, so much so that the proportion of water is less than in the ordinary bacterial cell.

Development of the Endospore. The essential part in the development of an endospore consists in a concentration of the protoplasm into a certain area, this area being then surrounded by a tough membrane. The rest of the bacterial cell usually disappears and the endospore is liberated. The first stage in this development is shown in Fig. 42*a*. The area into which the protoplasm condenses is visible by careful staining as a circular or oval area, which stains less deeply than the surrounding protoplasm. Some observers claim to have found inside this *vacuole*, as the area is called, a nucleus surrounded by a thin film of protoplasm, which is connected with the

Fig. 42.—Stages in development of endospore.

[1] De Bary divided bacteria into two kinds, those capable of forming endospores, and those incapable of doing so. In the latter case it was assumed that the vegetative cells themselves put on a thicker membrane and became resistant cells. In this condition they were called *arthrospores*. It was supposed that they afterwards germinated, but this has never been demonstrated.

periphery of the area by several strands of protoplasm. This is diagrammatically represented in Fig. 42*b*. In the next stage this space stains more deeply than the surrounding area. No doubt this is due to the fact that a large portion of the protoplasm has been concentrated into it (Fig. 42*c*). After this, the periphery of the young spore, as we may now term the area into which concentration is taking place, begins to take on a deeper stain, showing that a spore membrane is being formed (Fig. 42*d*). A stage, further in development, shows a still more definite membrane. In consequence of the presence of this membrane, spores take up stains very slowly and the ordinary staining methods never accomplish the staining of the contents of the spore (Fig. 42*e*). The last stage is a still further elaboration of this membrane, which is now seen to consist of two coats, an inner thin coat called the *intine*, and an outer tough coat, the *extine*, which often exhibits markings on its outer surface. The endospore is now mature. The remainder of the cell at the completion of this development is, in most cases, empty, the endospore being often seen lying free in an empty cell (Fig. 42*e*). In some cases, however, the protoplasm is not all used up in the formation of the spore, for motile individuals, each containing a spore, may be seen occasionally. It follows that in these cases there must be protoplasm inside the cell, for the cilia could not otherwise be functional.

Usually during spore formation there is no change in the bacterial cell (Fig. 42*e*), as, for example, in Bac. hirtus and Bac. anthracis, but in some species a swelling takes place at the point where the spore is being formed. Thus, in one of the bacteria which excrete butyric

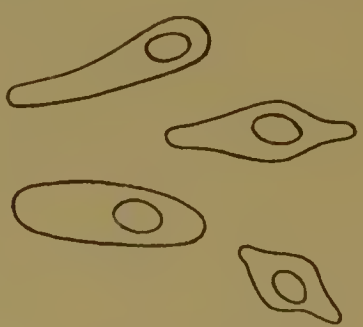

Fig. 43.—Bac. amylobacter.

Fig. 44.—Spore formation in Bac. tetani.

acid, Bac. amylobacter, the various forms shown in Fig. 43 may be seen during spore-formation. In Bac. tetani, which causes the lock-jaw disease, the swellings are invariably at one of the ends (Fig. 44), this characteristic being very useful for purposes of identification. Bac.

inflatus is also peculiar in this respect of swelling, for, as its name implies, the cell is much inflated and often two endospores may be found enclosed in one cell. Although usually one or two endospores represent the normal number, sometimes, when the cells become elongated, a large number may be present. In Bac. hirtus, the author has seen elongated individuals packed with endospores (Fig. 45). As

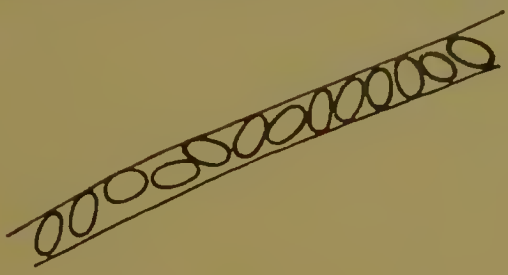

Fig. 45.—Spore formation in Bac. hirtus.

mentioned above, the formation of endospores has, so far, been observed only in two species belonging to the Coccaceae, viz. Sarcina pulmonum and Sarcina ureae. The development of the endospore has been followed in the latter, and in every particular it agrees with the above-mentioned development in the genus Bacillus. The power of resistance possessed by endospores may be judged by the fact that some, notably those of Bac. subtilis, can stand being placed for six hours in boiling water without being destroyed.

Structure of the Endospore. The endospore has two coats, the thin inner *intine*, and the thick outer *extine*. The latter is usually irregular

Fig. 46.—Spore of Bac. hirtus.

Fig. 47.—Types of spores. (*a*) Bac. asterosporus; (*b*) Sarcina ureae; (*c*) Bac. ruminatus; (*d*) Bac. graveolens, and (*e*) Bac. fusiformis.

on its outer surface, the commonest feature being blunt points, one at each end (Fig. 46). The endospores of Bac. asterosporous have elaborate markings, the extine being thrown into ridges and troughs as shown in Fig. 47*a*, whilst those of Sarcina ureae have six or seven

small blunt points (Fig. 47*b*). With regard to the size of these structures the following figures given by Gottheil for the spores of the soil-bacteria examined by him, will serve for this purpose:

	Length.
Bac. ruminatus, - - -	1·5–1·7 μ.
,, tumescens, - - -	2·5–3·0 μ.
,, graveolens, - - -	1·9–2·5 μ.
,, petasites, - - -	1·7–2·2 μ.
,, Ellenbachensis, - -	1·7–2·2 μ.
,, mycoides, - - -	1·4–2·2 μ.
,, subtilis, - - - -	1·7–1·9 μ.
,, pumilis, - - - -	0·94–1·52 μ.
,, carotarum, - - -	1·31–2·2 μ.
,, fusiformis, - - -	diameter, 1·3–1·8 μ.

The spores of Sarcina ureae measure 1–1$\frac{1}{3}$ μ in diameter.

Contents of the Endospore and Conditions of Spore-formation. The smallness of the spore and its thick, almost impenetrable outer membrane, make it impossible to investigate the contents very closely, but we are sure that the spore, like all similar structures, will contain dense protoplasm, and enough food material to give it a start in germination; for, given the necessary moisture and temperature, spores will germinate on a barren medium, *e.g.* a jelly made of gelatine and water only. A large number of bacteria never, so far as we know, form endospores, but it is doubtful whether they are never under any circumstances able to form them. Many investigations have been conducted with the aim of ascertaining the conditions which influence spore-formation. In the case of Bac. inflatus it has been found that a 1·2 per cent. solution of meat extract in the nutrient medium has a favourable influence, whereas grape sugar has quite the opposite effect. Another and anaerobic species (Clostridium butyricum) forms endospores only when growing in an atmosphere devoid of oxygen, while, on the contrary, Bac. anthracis, Bac. subtilis and several others form these spores only if oxygen is present. The author has found that a very slight percentage of acid influences unfavourably the formation of spores in the case of Sarcina ureae. No one has yet, despite many attempts, been able to cause a species to form spores, if the species does not normally do so, though on the other hand it is not difficult to prevent spore-formation in a species which normally forms spores.

Most sporogenous species, growing on solid nutrient media, commence to form spores on the second day after inoculation, though in

liquid cultures, spore-formation is sometimes very active after twenty-four hours. Again, if the conditions are not favourable, it may be postponed for several days, when spores will be found in greatly reduced numbers, as the majority of the individuals, under unfavourable circumstances, seem to have lost the capacity of producing them.

Not much can as yet be said with regard to the germination of spores belonging to the order Coccaceae. The spores of Sarcina ureae are round and possess two coats, the inner thin, the outer thick, resistant and studded on the outside with six or seven blunt points (Fig. 48). During germination the spore first loses its highly refractive appearance as a result of the swelling which it undergoes. Fig. 48 shows the subsequent changes that take place. The

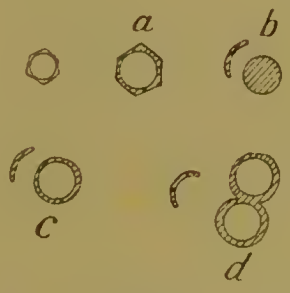

Fig. 48.—Germination of spore of Sarcina urea.

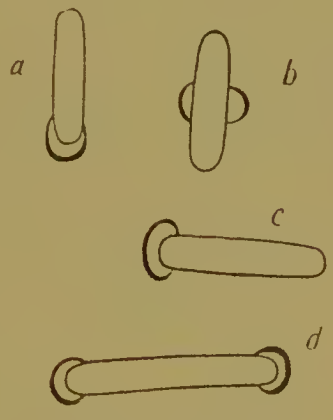

Fig. 49.—Types of germination.

outer membrane is burst open and the contents emerge clothed in the inner membrane. The young coccus, after freeing itself from the outer spore-coat, increases in volume, and surrounds itself with a more substantial outer membrane (Fig. 48*c*). In some cases division rapidly follows, as seen in Fig. 48*d*, where the outer spore-coat is still in the immediate neighbourhood. The time required for germination is the same as for bacillus-spores, viz. between four and six hours.

With regard to the genus Spirillum, spore-formation and spore-germination have been recorded, but neither process has since been observed, and the figures are not trustworthy, so until the results of more accurate research are forthcoming, we must withhold our judgment as to whether the spiral bacteria form spores.

Spore Germination. We have seen that an endospore has two coats. In germination the outer is left behind, but the inner

very probably becomes the membrane of the new vegetative cell, though the latter fact has not been demonstrated. Germination can be very easily observed in the case of those rod-shaped forms which sporulate freely on nutrient agar. The method adopted for observation is as follows: The material is added very thickly to a drop of sterile water, then heated for two minutes at 100° C. to kill off all the vegetative cells, and then sown very plentifully on the surface of an agar-slope. The inoculated tube after being placed in an incubator (28-32° C.) for about six hours, is taken out and microscopically examined. It is advisable to stain with weak fuchsin. The first process consists of a swelling of the spore, with a concomitant loss of its bright glistening appearance. This is evidently the result of absorption of water from the surrounding medium. Then follows a split in the extine through which the young cell protrudes by elongation. This protrusion can be effected in three distinct ways:

(1) Polar. When it emerges from one of the poles (Fig. 49*a*).

(2) Bi-Polar. When it emerges from both the poles (Fig. 49*b*).

(3) Equatorial. When it emerges from the middle of the spore (Fig. 49*c*).

Examples of polar germination are seen in Bac. graveolens, Bac. Ellenbachensis, Bac. mycoides, etc.; of equatorial germination, in Bac. subtilis, Bac. hirtus, Bac. petasites, etc.; and of bi-polar germination in Bac. simplex, Bac. cohaerens, etc. Some bacilli germinate in two of these ways, *e.g.* Bac. cohaerens is at first polar in its germination, then at a later stage the germinating cell may also break out at the other pole, and so become bi-polar. The spores of Bac. subtilis have a further peculiarity in that they occasionally burst the spore membrane right round, so that the halves separate and the young vegetative cell carries a bit of the spore membrane at each end (Fig. 48*d*). The time required for germination varies according to the medium. Prazmowski records 3-4½ hours at 30°-35° C. for Bac. subtilis. This is doubtless correct for the majority of species, because, if the culture be examined six hours after sowing, it is usual to find a large number of germinated spores, showing that the first spore must have germinated at least a couple of hours sooner.

Conidia- and Gonidia-Formation. Among the higher bacteria (Chlamydobacteriaceae) the formation of endospores is unknown, but instead, other kinds of spores are formed. There are two kinds, called respectively *conidia* and *gonidia*. Both differ from endospores in being less resistant, in being produced in far greater numbers,

and in having a different mode of development. They also differ from each other in their mode of development.

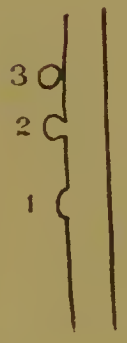

Fig. 50.—Leptothrix ochracea. Showing mode of development of conidia.

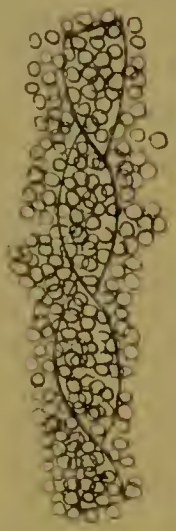

Fig. 51.—Spirophyllum ferrugineum. In state of active conidia-formation.

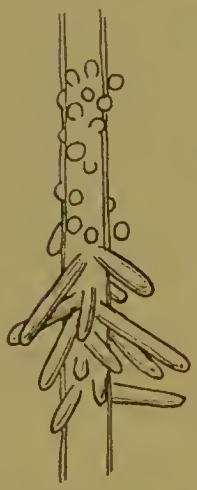

Fig. 52.—Leptothrix ochracea. Showing conidia germinating *in situ*.

Conidia (sing. conidium). These are best seen in some of the iron-bacteria, notably Leptothrix ochracea and Spirophyllum ferrugineum. The mode of their development is shown diagrammatically in Fig. 50. A small wart-like protuberance emerges from the thread, which, after it has attained a certain size, is cut off by constriction. The small body thus cut off is called a *conidium*. When one has been separated off, another is formed in the same place. As under favourable circumstances conidia are forming all over the organism, and as the conidia after separation do not move away, it often happens that the organism itself is invisible, being buried beneath thousands of conidia (Fig. 51). These bodies are obviously formed for purposes of rapid multiplication. Like the conidia that are produced

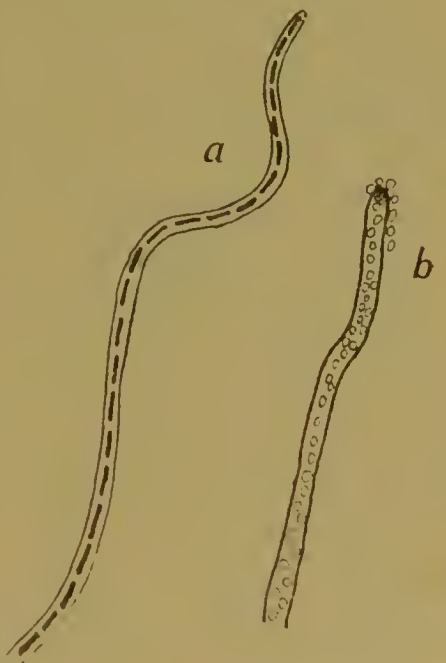

Fig. 53.—Chlamydothrix hyalina. (*a*) Before gonidia-formation; (*b*) during gonidia-formation. (After Migula.)

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by moulds, such as Penicillium or Eurotium, they are capable of germinating immediately after their formation. This is shown by the fact that Leptothrix threads are sometimes seen with small threads

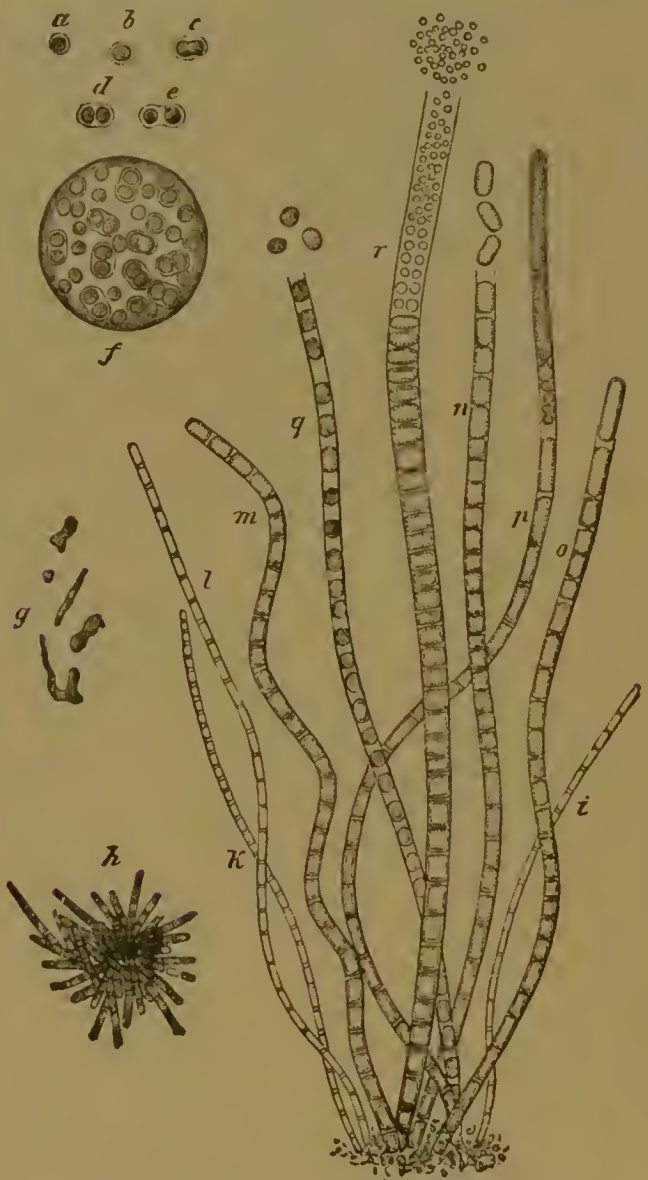


Fig. 54.—Crenothrix polyspora. (After Zopf.)

attached to them. These have resulted from the germination of the conidia whilst still attached to the parent organism (Fig. 52). As to their method of germination, in the case of Spirophyllum ferrugineum, the membrane of a conidium bursts open, then the contents emerge and develop by growth into a new band. On the other hand,

it is very probable that a conidium can also elongate directly into a new individual.

The size of the conidia in the various species which form them is remarkably constant, being about $1\frac{1}{4}\,\mu$ in length and $1\,\mu$ in breadth.

Gonidia (sing. gonidium). These differ from conidia in their mode of origin, being formed usually by the breaking up of cells, each into a number of smaller ones, which are termed *gonidia*. Fig. 53*a* represents a thread of Chlamydothrix hyalina before gonidia formation has set in. Fig. 53*b* shows the same kind of thread full of gonidia.

Crenothrix polyspora, one of the iron-bacteria, forms gonidia of two sizes, named respectively *macrogonidia* and *microgonidia*, the former being the larger and the latter the smaller ones (Fig. 54). The gonidia are thrust out of the thread and develop each into a new thread by a process of elongation (Fig. 54*h*). Occasionally they develop whilst still attached to the parent organism.

In *Cladothrix dichotoma*, another of the iron-bacteria, the gonidia assume a more highly developed form, for they are comparatively large and each is supplied with a lateral bundle of cilia (Fig. 15). By means of these cilia, the gonidia swim actively away, finally attaching themselves to a suitable support. Here they get rid of their cilia and develop into new threads.

CHAPTER III.

§ 1. VARIOUS GROWTH-FORMS OF BACTERIA.

As described in Chapter II. multiplication usually takes place in bacterial cells by division, followed by separation of the daughter-cells, which in turn repeat the process of division and separation, until a vast number of individuals has been produced. If, however, no separation takes place after division, chains of cells are formed in the case of the rod-bacteria, and in the case of the cocci-species we have seen that chains (Streptococcus), plates (Micro- and Planococcus), packets (Sarcina and Planosarcina), or irregular groups (Staphylococcus) may result. These modes of combination are not invariably fixed for any particular species, except in the case of the Streptococci species, which produce chains, and chains only. On the other hand, a Sarcina species can form packets, irregular groups, plates, and sometimes the culture may consist of groups of from one to four only in each group. Growth-forms similar in nature to the above are absent from the Spirillum species.

An important growth-form is the *pellicle* or skin which forms on the surface of the liquid in which bacteria are growing. This pellicle consists of millions of bacteria held together by their membranes, the whole forming a comparatively thick white, or grayish-white skin of firm consistency on the surface. This formation takes place only in some species of bacteria, *e.g.* Bac. subtilis, and Bac. fluorescens-liquefaciens, and is an important aid in the diagnosis of those species which form the *pellicle*.

In some species this banding together of individuals takes place *under* the surface of the nutrient liquid, and in irregular masses. This is called a *Zoogloea*. In this condition the bacteria are better able to resist unfavourable circumstances. This formation is of common occurrence among other low forms of vegetable life. The zoogloea

consists of a structureless, irregular lump of jelly containing bacteria inside it, and its surface may be either smooth or folded. In nature, a zoogloea may be formed from individuals of one species only (homogeneous zoogloea), or several species may have contributed to its formation (heterogeneous zoogloea). When the mucilaginous material is liquefied, the imprisoned bacteria assume once more their normal activities. This condition must be regarded as one in which the formation of mucilage has been much more active than cell-division.

The best known example of the zoogloea condition is that of Leuconostoc mesenteroïdes (now called Streptococcus mesenteroïdes). This coccus forms huge slimy masses in nutrient solutions which contain sugar, and much damage has been done by it in sugar mills, for in the sugar solution, before it is crystallised and refined, this organism finds a most suitable medium. The cocci are formed in chains (Fig. 55), hence the original name Leuconostoc has now been dropped and that of Streptococcus substituted.

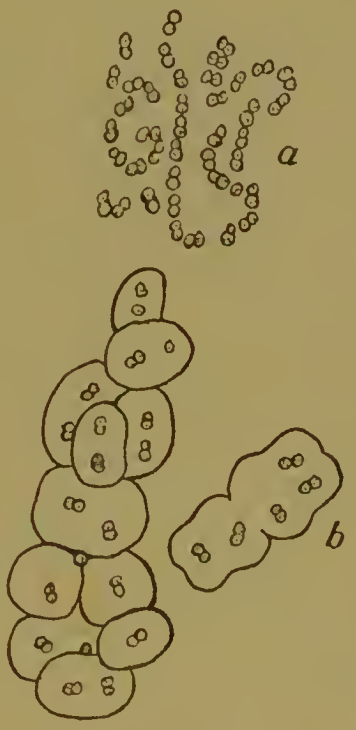


FIG. 55. — Streptococcus mesenteroïdes. (*a*) Appearance when mucilage is not developed; (*b*) zoogloea condition.

Another well-known case is that of the Kefir grains. These bodies, when placed in milk, cause fermentation to take place, with the result that a small percentage of alcohol is produced. A Kefir grain is a lump of mucilaginous matter consisting of several kinds of organisms, chiefly bacterial, and is therefore a heterogeneous zoogloea.

Much attention has been given to the question of the existence of *Pleomorphism* among bacteria. This term refers to the capacity whereby a single species is enabled to assume different forms. Among the fungi, the Uredineae and Ustilagineae show very marked pleomorphism. The various forms of one of these plants are so different that it is difficult to believe that they all belong to the same species. Among bacteria, however, pleomorphism of the same kind as is found in the above-named fungi does not exist. What we do find in some species is a remarkable variety in the forms that may be assumed by

individuals of the same species. This, however, is not pleomorphism, but simply morphological variations which are found more or less in most bacteria of the rod variety, and in two species which change alcohol to acetic acid, viz. Bac. aceti and Bac. Pastorianum, most remarkable structures have been figured by Prof. E. Chr. Hansen (Figs. 56 and 57). These varieties of form, however, depend on the

Fig. 56.—Bac. aceti. (After Hansen.)

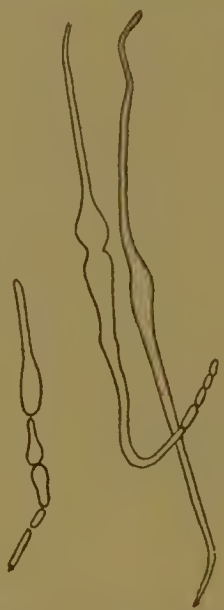


Fig. 57.—Bac. Pastorianum. (After Hansen.)

environment, and do not represent definite phases in the life-history of the species in which they are exhibited. The exact nature of the causes influencing the formation of morphological variations has not been sufficiently studied, but the following are probably among the number:

1. The constitution of the nutrient medium in which the species is growing.
2. The nature and amount of excretion products.
3. The presence of definite chemical substances.

Finally, among growth-forms mention must be made of what are known as *involution* structures. These are unhealthy structures, and when they occur it is a sign that the species is growing under unfavourable conditions. Under involution forms are included all irregular structures, such as unnatural swellings, branchings and distortions. The best known are those which occur on the nodules that are found on the roots of plants belonging to the Leguminosae (Pea

and Bean family). These belong to a species called Bacillus radicicola, and the mode of their production will be discussed when we come to treat of the Nitrogen bacteria to which they belong. The normal form is that of a short rod, but the involution forms are like those exhibited in Fig. 58, in which we see that some of the cells are branched whilst others exhibit irregular swellings. Fig. 59 represents Spirillum giganteum showing this feature, and other examples may be easily seen in Bac. tuberculosis, Bac. diphtheriae, and a large number of other bacteria. That they are unhealthy structures may be demonstrated by staining with methylene blue or one of the other stains of this class, when it will be seen that the inside of the involution forms are only faintly or not at all coloured, showing absence of

Fig. 58.—Bac. radicicola. Involution forms.

Fig. 59.—Spirillum giganteum. Involution form.

protoplasm. Again, if the species is one like Spirillum giganteum that shows a large quantity of reserve material, it will be found that the involution forms are almost entirely devoid of this material. They never divide or show any signs of life, and may be regarded as individuals which are either dead or dying. There is no doubt that the line separating structures included under morphological variations and involution forms, is not sharply defined. Whilst the researches of Hansen on Bac. aceti and Bac. Kützingianum have shown that in these species fantastically shaped cells were not unhealthy, for division took place in them, in the majority of forms it may be assumed that distortion and unhealthiness go hand in hand.

§ 2. PHYSIOLOGICAL VARIATIONS.

The descriptions of the variations in the first paragraph of this chapter were concerned with changes of form. There are, however, bacteria, which, although belonging to the same species, and outwardly quite similar, show a great variety in their physiological characteristics, that is, their functions are not all alike. Thus, one culture will liquefy gelatine, another culture of the same species, with a different past, may

have lost this capacity; one culture produces a red pigment, another has not the power to do so, and so on. We could multiply similar instances indefinitely. This will be understood more clearly when it is remembered that precisely the same variations are found among mankind. Thus, one man having been brought up in such a way that his system has been hardened, can stand an amount of exposure that would kill another man who had been differently nurtured, and yet outwardly these two men might appear very much the same. Bacteria are very sensitive to their environment, and great changes can be effected in them in a comparatively short time, without their external appearance being altered. They are probably in this respect more sensitive than any other organisms. This being so, it can be readily understood that bacteriologists look forward to the time when the energies of these organisms can be directed far more than at present towards furthering the well-being of mankind.

The physiological variations of bacteria have been responsible for much confusion in their nomenclature, and there can be no doubt that of the thousand odd species of the genus bacillus, a large number to which names and descriptions had already been given have been described and named anew, owing to differences under different conditions. The greatest variability is seen in the colours of cultures of bacteria. Sarcina aurescens, for example, has been known to assume such different colours as light orange, yellow, or gray, when cultivated by different observers. The exact nature of these variations is unknown.

Again, a species normally motile does not always exhibit motility. As the motility is due to the lashing of the cilia, the absence of motility is due to one or other of the conditions which prevent the development of the cilia, or cause them to be thrown off when developed, or to conditions which cause the development of an undue amount of mucilage round the cell-walls.

Again, it is possible, within a comparatively short time, to effect the formation of a new physiological variety with regard to heat, by submitting a particular species to a process of artificial selection. Cultures of a species are submitted to different degrees of heat, the "breeding" for the next generation being effected from that culture which has suffered most heat without destruction. Thus, suppose that a series of six culture-tubes, after inoculation with a particular species, were heated respectively for 1, 2, 3, 4, 5 and 6 minutes at 100° C., and suppose that 1, 2, 3 and 4 showed growth, while 5 and 6 did not. The next series would be selected from the number 4 culture, which, of those in which

growth had taken place, had stood the greatest amount of heat [1] When this process has been continued for some months it is possible to obtain a culture, the individuals of which are quite similar in appearance to those in the original culture, but different from them physiologically in being able to stand a greater amount of heat without having their vitality impaired. The instances of physiological, unattended by morphological changes, could be indefinitely multiplied, but want of space precludes a fuller discussion of the subject in this book.

From the above remarks it will be seen that great care must be taken in the diagnosis of species, and we must not necessarily refuse to believe in the identity of two species merely because they differ in one or two characteristics. The method followed by bacteriologists is to study the life-history of a species in all its aspects, and examine it under different conditions before passing judgment as to its position or name. Only in this way, by judging of the sum-total of characteristics, is it possible to eliminate mistakes arising from physiological variations.

§ 3. RELATIONS OF BACTERIA AMONG THEMSELVES.

The complicated relations of life are nowhere more manifest than among bacteria. It must be borne in mind that bacteria are constantly eating up food-material, and what is more important, constantly excreting their waste matters, which are usually poisonous to themselves and to other bacteria. Then their power of reproduction, when conditions are favourable, is so rapid that if, in any particular nutrient medium, one species is slightly more favoured than the others, this produces such a large number of individuals that the others are either swamped out of existence or are present in very small proportionate numbers. If, however, the conditions change ever so little, and the change is more favourable to one of the weaker organisms, this in its turn multiplies so rapidly that often after a comparatively short time it appears to be the only organism present. This, again, may be replaced in predominance by a third form. That changes are continually taking place is evident, when we reflect on the injurious excretion products that are being continually formed; some of these

[1] When growth has taken place after the culture has been subjected to heat, it must not be imagined that *all* the individuals in the culture have resisted the heat. What has happened is that all except a few have been destroyed, but that the multiplication of these few has made good the loss.

are of an acid nature, which act more injuriously on some bacteria than on others. Again, changes in the gaseous constitution of the environment, especially in the amount of oxygen present, affect different bacteria in different ways. To these may be added changes in the temperature, in the degree of moisture and other factors. Hence we may assert that where bacterial growth is taking place, the environment is continually changing. In the early days of Bacteriology many mistakes occurred, because the conditions of bacterial growth, and especially the relations of the growth to the environment, were only imperfectly understood. It was frequently asserted that any particular species assumed several forms in the course of its life-history, that, for example, a bacillus, under certain conditions, assumed the coccus-shape, and under other conditions even a spiral-form. From what has just been stated it will be seen that the investigators who made these statements had not worked with pure cultures, and that the apparent change from one shape to another was really due to the predominance first of one organism and then of another of a different shape.

A good example is given us by one of Lister's experiments. Ordinary milk was allowed to become sour spontaneously. A drop of the sour milk was introduced into boiled milk, another into sterilised beef extract, and a third into sterilised urine. After growth had taken place inoculations were made from each into Pasteur's nutrient solution, then from this to urine, and finally back again into milk. Assuming that the souring of the milk had been effected by one organism, Lister concluded that the various forms found in the resulting cultures were all descendants of one species, which had undergone strange changes in form as a result of having been grown in different media. The explanation is seen when it is remembered that the sour milk from which he started contained several organisms, and in each medium a different one became predominant. These results caused Lister to give the name of Bacterium lactis to what he thought was one species. This mistake went so far on the Continent that one investigator declared that all bacteria were modifications of one species which he called Coccobacteria septica.

It does not always happen that two organisms feeding on the same medium are so antagonistic that one has to give way. A good example of this is Bacillus coli communis. Although normally present in the alimentary canal, it produces no evil effects; in conjunction however with other organisms it is able to set up acute intestinal irritation and produce various changes of an inflammatory nature in the body.

The state of matters in which two or more organisms work hand in hand, each helping the other, is called *Symbiosis*. Other examples of symbiosis are the association of Streptococcus and the bacillus causing diphtheria, Bacillus coli and yeasts, the bacillus causing lock-jaw and putrefactive bacteria, and the association between Diplococcus pneumoniae and Proteus vulgaris. This subject is very little understood, but its importance cannot be overestimated, especially the conditions of symbiosis of the pathogenic bacteria with other organisms. Thus it does not follow that because a certain pathogenic microbe is present in our bodies, we suffer from the disease in question. We suffer only if this microbe multiplies, and multiplication takes place only if certain conditions hold. It is important that we should know more of the organisms which tend to bring about these conditions. The Caucasian drink called Kephir is an interesting result of symbiosis. This is an effervescent, alcoholic sour milk prepared from the milk of sheep, goats, and cows. The method of its manufacture is simple. A few "kephir grains" are added to the milk, which is then allowed to stand for 24 hours at room temperature, then poured off to allow fresh milk to be added to the grains. Fermentation is complete in two or three days, the mixture containing about two per cent. of alcohol. The "kephir grains" are a mixture of three organisms, a filamentous bacillus forming "zoogloea," a lactic-acid producing bacillus, and a yeast. These three organisms are obviously in a state of symbiosis, though what the exact nature of it is, is very difficult to say.

Another well-known instance is the so-called ginger-beer "plant," which is the agent in the production of stone ginger-beer. These "plants" consist of two organisms, a yeast called Saccharomyces pyriforme, and a bacillus called Bacterium vermiforme. The production of ginger-beer will be described in a later section, it is sufficient here to notice the symbiosis of the two organisms. Another interesting case is that of the two yeasts, Saccharomyces pastorianus III., and Saccharomyces ellipsoidens II, which, according to E. Chr. Hansen, acting together, cause turbidities and other beer diseases, though each alone is harmless.[1]

[1] The importance of a knowledge of symbiosis, especially to the practical man, is seen by the results of Nencki's experiments. The bacillus of symptomatic anthrax in nutrient solutions containing cane-sugar, produces the following substances as fermentation products: hydrogen, carbon-dioxide, normal butyric acid, and active lactic acid. On the other hand, micrococcus acidi paralactici in the same medium produces almost exclusively optically active paralactic acid. Now, when cultivated together in the same nutrient solution, in addition to the above-mentioned substances, normal butyl-alcohol is produced.

Sufficient examples have been given to demonstrate that among bacteria and their allies, cases of mutual help are not uncommon, and later investigations all tend to show that such associations are frequent throughout the whole of the vegetable kingdom, though at present our knowledge of the *modus operandi* is also in this branch very slight.

We must now notice another relation subsisting between various kinds of bacteria, viz. the cycle of events by which organic matter is brought into a condition in which it is available for plant absorption. The changes that take place can be represented by a series of steps (Fig. 60).

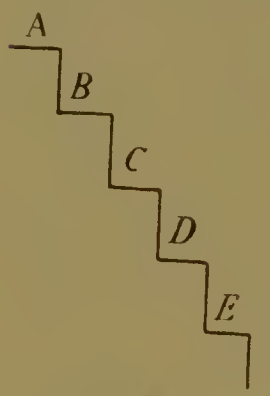


Fig. 60.
(For explanation see text.)

Suppose *A* represents some organic matter, *e.g.* the body of an animal which has just been killed. A certain microbe fastens on it, and, multiplying very rapidly, changes the body into a substance *B*. It is now ready for a second microbe which feeds on *B*. The first microbe has either died off or gone into the resting condition, and the second microbe now becomes predominant, and changes the body into a third substance *C*, and it, in its turn, is displaced by a third germ which has the power of feeding on *C*. By a series of steps the body becomes changed into substances utterly unlike what it originally consisted of. This relation by which the food of one kind of bacteria is prepared by the activity of another, is called *Metabiosis*. It must not be thought, however, that the process of decay of any organic body is as simple as that just described, for several kinds of bacteria are usually present, in which case, either they help one another, producing a state of symbiosis, or they may be antagonistic, producing a state of *Antibiosis*, in which the changes wrought by one kind act as a poison to another kind which is feeding on the same material. In the decay of organic remains, symbiosis, metabiosis, and antibiosis, are all more or less in evidence.

We may represent what takes place in the following manner:

A is the organic material; 1, 2, 3, 4 are the bacteria feeding on it. 1 changes *A* into three substances p, p', p''; 2 into q, q', q''; and so on. If, now, p' is poisonous to 2, then 1 and 2 are antibiotic. If p'' is conducive to the growth of 3, then 1 and 3 are symbiotic. If 5 feeds on s' changing it into t, t', t'', then 4 and 5 are metabiotic. A state of antibiosis can also be obtained when two organisms, not necessarily

injurious to each other, feed on the same substance, for each takes food away from the other.

By means of these changes all organic matter is made ready for reabsorption into the bodies of plants, and later, as animals feed on plants, into the bodies of animals. In this way putrefactive bacteria act as true philanthropists, because, by their activity, the accumulation of dead organic matter is prevented. Also, if the organic matter were not changed, there would result a dearth of food for the plants, and ultimately the extinction of all plants and animals would follow. The exact method by which aid is given to plants cannot be explained until we have considered the sulphur-, the nitrite-, and nitrate-bacteria.

§ 4. METHODS OF PURE CULTURE.

A pure culture means a growth in which only one species is present. Before 1878 pure cultures were unknown, so that descriptions of species before this date are unreliable, as errors were made because cultures apparently consisting of one species were really mixtures of several kinds. The result was that the characteristics of several were described as those of one species. In 1878, however, Lister devised a method for isolating the various kinds of bacteria from a mixture containing several species. This is called the *Dilution method*, and it consists in so diluting the liquid containing the bacteria that a small portion extracted from it by means of the platinum loop or wire will contain only one kind. When the portion thus extracted is now transferred to a sterile nutrient tube the resulting growth will consist of the derivatives of one species only. Lister was thus the first to obtain a pure culture. Pure cultures of yeast were first made by E. Chr. Hansen by this method, a fact for which the brewers of the present day have great reason to be thankful.

This method is very seldom employed nowadays, as it is somewhat cumbersome in comparison with the *Plate method*, and will separate out only one form, whereas the plate method will separate several in one experiment.

A second method is that called the *Fractional method* of culture. In a culture containing a mixture of bacteria one form will predominate, and consequently be more numerous. When this is at the zenith of its activity an inoculation is made from this culture to a second. In this way the predominant bacteria start with an advantage over the other competitors. As it is better adapted for this culture medium—

as shown by its predominance—it will tend to become still more predominant in the second culture. An inoculation is now made from the second into a third nutrient tube. This still further intensifies the predominance of the predominant form, so that after three or four such inoculations we may reasonably hope to obtain a culture in which all the other forms have been eliminated. This method has, like the first, the disadvantage of permitting only one form to be isolated, and it also requires a longer time, for we have to wait till the culture has reached its zenith of activity, which may take two or three days, and certainly not less than one, so that when often repeated much time is lost. Care has to be taken that all the tubes contain exactly the same nutrient medium, otherwise the new conditions may be more favourable to the species very feebly represented, in which case these latter will soon become predominant, and the experiment be valueless.

Koch's Plate Method is now universally employed. This method enables us to separate several species at once, is easy to perform, and when done with Petri-dishes, is considerably better than the other methods in every respect. We shall therefore describe it in detail.

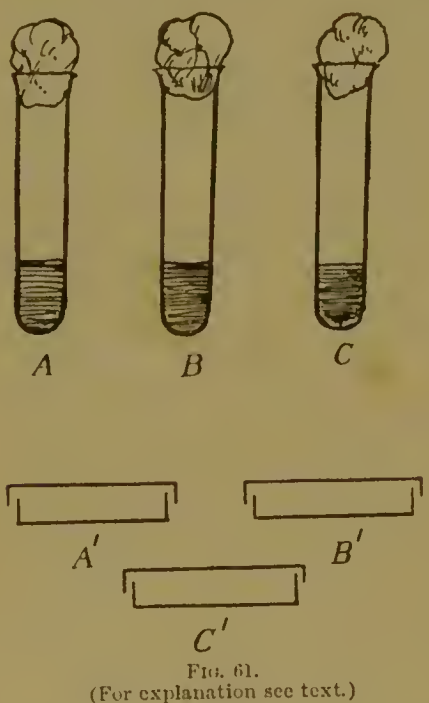


Fig. 61.
(For explanation see text.)

Three sterilised tubes, each containing about 8-10 c.c. of sterilised nutrient gelatine, are gently heated in order to liquefy the gelatine. Let us call them *A*, *B*, and *C* (Fig. 61). A small quantity, as much as a platinum loop will hold, is removed from the liquid under examination, care being taken to sterilise the platinum loop in order to exclude all extraneous bacteria. The platinum loop is dipped into the tube *A*, while the gelatine is still liquid, taken out and again sterilised by passing through the flame. After *A* has been gently shaken, the platinum loop is inserted into it and the same quantity of liquid removed from *A* to *B*. The process is repeated so that a loopful is transferred from *B* to *C*. The effect of this process on the distribution of bacteria in the three tubes is as follows: *A* will contain thousands of bacteria, and if we are examining a liquid like sewage-water, it will

contain millions. Now, since only a loopful is taken from *A* for the second inoculation, it follows that the number will be reduced to hundreds only. When repeated from *B* to *C*, the latter will contain only a very small number, perhaps about ten or less. The process so far is thus practically a dilution method. Three sterilized Petri-dishes (Fig. 61, *A'B'C'*) are next placed in a row on the table. A Petri-dish is a round glass dish from 6-10 cms. diameter, with glass sides about 1 cm. high. It has a glass cover which, as is seen in the figure, overlaps the sides of the dish. The contents of the now inoculated gelatine tubes are poured each into a separate Petri-dish, where the gelatine cools and solidifies into a jelly. The three Petri-dishes are now put away inside a glass dish covered by a bell-jar, and at the bottom of this dish a thin solution of copper sulphate is usually placed to keep off the moulds (Fig. 62). As the bacteria are encased in the Petri-dishes, and these in their turn are encased in a glass house, no bacteria from the outside can participate in the food present in the nutrient gelatine. Now consider what will happen. Each cell will divide, the process of division being carried on by the daughter-cells, so that in a day or two there will be an immense number of individuals present. But the case is different from that of a liquid culture. Here the products of one cell remain stuck together on account of the medium being solid, therefore the gelatine, after about two days, will be seen to be punctuated with a number of small dots, each dot consisting of the descendants of one cell, and probably containing about a million individuals. The Petri-dish which received the contents of the gelatine-tube *A* will usually be found to have so many of these dots that separation will be impossible. The same is often the case with *B*. In *C*, however, the state of affairs is different. Suppose that ten bacteria were contained in the gelatine, they are poured out along with it into the Petri-dish. The gelatine just covers the surface of the glass in the Petri-dish, and should not be more than 1 mm. thick. Of course these ten are invisible, but when each one multiplies to such an extent that where one was present there are now a million, and when the million stick together, the sum total becomes visible. If they all develop, ten spots, usually round or oval, will be seen on the surface of the gelatine, or perhaps some on the surface, others under it. These dots are called *Colonies*. The next process is to touch a colony with a sterilised

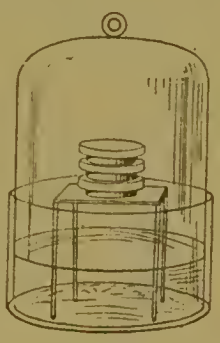


Fig. 62.
(For explanation see text.)

platinum needle, and inoculate an agar-nutrient-tube. This is placed in a cupboard at room temperature, or, when growth is slow, in an incubator. In about 15 hours, owing to the bacterial growth, the surface of the agar will be covered by many millions of the organism. They appear to the naked eye as a transparent or opaque and coloured covering, according to the nature of the species. There is a good deal of variation in the nature of this growth. The chief point to note at present is the fact that all these millions of individuals are derived from a single individual, therefore this is a *pure culture*. If the study of this microbe is desirable as being of importance in any branch of industry, or the cause of some disease, that study can now be carried out, and the characteristics of the species accurately observed without any complications due to the presence of other bacteria. Our knowledge of such diseases as consumption, lockjaw, diphtheria, etc., is now widely extended; and that is due to the fact that the effects produced by the micro organisms in these diseases have been carefully studied by the aid of pure cultures. Among yeasts, the isolation of the various species, by Hansen and others, has enabled brewers to work with pure cultures when transforming the wort into beer. In working with mixed yeasts a brewer is never certain when a predominance of "wild" yeasts may occur. If they gain the upper hand in wort, the loss to the brewer is considerable. Finally, we may mention the use of pure cultures for the souring of cream, preparatory to churning, in the production of butter. It is to be hoped that other fermentative industries will, in course of time, be able to utilise pure cultures, instead of, as at present, relying on chance organisms that are present in the atmosphere, to produce the desired effects.

§ 5. METHODS OF EXAMINATION OF BACTERIA.

The identification of bacteria is not a simple matter, for, first, there are so many organisms with similar appearances and of an approximate size and shape, on the surface of nutrient media; and, secondly, there is so much plasticity in a bacterial growth that the same species under different conditions can never be relied upon to have the same characteristics in all the cultures. Sarcina mobilis may serve as an instance of this plasticity. In the cultures of Maurea, by whom the species was discovered, the growths were brick-red in colour. Migula, who obtained his culture from Maurea, states that his cultures were orange-yellow. In the author's cultures, obtained indirectly from Migula, the growths

were at first grayish and finally lemon-yellow. All these cultures came from the same source and yet we have four colours represented. In the same way it has been observed by bacteriologists that colour-forming bacteria often lose this power by being grown for several bacterial generations in laboratory cultures. Still another example of their plasticity is the change in size which the individuals undergo when their environment changes. When circumstances are unfavourable, they not infrequently diminish very much in size. This is usually the precursor of death, but not always, as sometimes a protracted existence can follow after a partial adaptation has taken place. Instances can be multiplied, but these are sufficient to show that, in the study of any species, reliance must be placed on the sum total of characteristics, and a knowledge of the whole life-history. The following methods of cultivation and observation are those most commonly used:

1. **Gelatine Plate-culture.** The method of procedure is that described under the section dealing with the methods of obtaining pure cultures The colonies are examined and the following points noted:

(1) Colour, size, and shape of colonies.
(2) Rate of growth.
(3) Whether growth is on surface, under surface, or both.
(4) Whether liquefaction takes place.
(5) Microscopically the size, shape, and motility of the cells.
(6) Microscopically the appearance of the edge of the colony and the various modifications of form assumed by it.

It will be found that No. 6 needs careful attention, as the appearance of the different colonies of the same species, even in the same plate, is often very various, sometimes leading to the impression that more than one form is present.

2. **Agar Plate-culture.** Prepared in the same way. It has the advantage that plates can be placed in incubators at fairly high temperature, and so more rapid growth is obtained. It has the disadvantage that growth over the surface takes place very rapidly, so the plates are soon spoiled for observation purposes. The points to be noted are those given above for gelatine plate-cultures.

3. **Gelatine Stab-cultures.** A test-tube is filled with about 6 cms. of nutrient-gelatine, which, when solid, is stabbed with a platinum needle which has been dipped in a solution containing the species in question. The following points are noted:

(1) Whether growth on surface only, along the stab only, or on both.

In the case of aerobic bacteria, viz. those that can grow only when oxygen is supplied to them, growth will take place at the surface. In the case of anaerobic bacteria, which cannot thrive when supplied with oxygen, growth will take place only along the stab.

(2) Colour, size, and shape of growth on surface.

(3) Whether liquefaction of gelatine takes place. This takes place in various ways; these should be carefully noted.

(4) If liquefaction takes place, the sediment must be noted, its colour, and amount of deposit. Also, if the sediment is absent, whether there are any particles held in suspension in the liquefied gelatine.

(5) Whether gas is developed. This is a valuable characteristic when it occurs. Thus, by this means, Bacillus coli communis is easily distinguished from Bac. typhosus, although similar in several other respects.

(6) Microscopically the size, shape, and motility of the cells.

(7) If growth is along the stab, whether indicated as a continuous line, or as a series of dots.

4. **Gelatine Shake-cultures.** Prepared in the same way as No. 3, only inoculation is effected when the nutrient-gelatine is in a liquid state. The same points are to be noted as in No. 3.

5. **Agar Slope-culture.** The agar is allowed to solidify in the sloping condition (Fig. 63). There should always be a small amount of water at the bottom of the slope. The bulk of the observations are usually made on this form of culture. These points are noted:

(1) Colour of growth on surface.

(2) Size, shape, and motility of the cells after 6 hours, 15 hours, 24 hours, 48 hours, 72 hours, 5 days, and 10 days growth on the nutrient-agar. If the material consists of spores only, the life-history from spore-germination to spore-formation will usually be traversed in about 3 days, but under different circumstances this period may be prolonged or shortened. The whole life-history may be passed over in one day under certain external conditions at present unknown.

(3) Any changes in the condensed water at the bottom of the slope.

(4) Any special peculiarity in growth, whether thick or thin or mucilaginous. These, however, are not of much value because of the extreme variability of growths.

(5) Presence or absence of fluorescence.

(6) Presence or absence of a pellicle extending from the agar surface over the surface of the condensed water, *e.g.* Bac. subtilis, the Hay-bacillus, invariably shows a thick pellicle.

6. **Growth in Various Liquid Nutrient Media.** The bacteria require to be supplied with their nitrogen and carbon in different ways. The usual medium employed is a mixture made up of Lemco, common salt, peptone and water, which mixture suits the needs of almost all bacteria. To obtain a complete knowledge of the organism, it should be grown in different liquid media, in which the nitrogen and carbon supplies are different. The table on next page, taken from Fischer's *Vorlesungen über Bakterien*, illustrates the aid given by these cultures to the diagnosis of the species.

FIG. 63.—Agar slope-culture.

The results of this set of experiments are quite sufficient to show that this method of cultivation is valuable as an aid to the diagnosis of species. In this connection it must be noticed that the effect of the excretion products is sometimes of an acid and sometimes of an alkaline nature. The same organism may induce acidity in some culture-liquids and alkalinity in others. Bac. hirtus is an example of this kind.

7. **Treatment with Stains.** It is always advisable to treat with stains in order to ascertain whether any cell-contents are perceptible, and if so, to determine their nature. Thus Spirillum gigauteum, Bac. tumescens, and others have fat globules, Bac. asterosporus has glycogen, and Spirillum gigauteum and Bac. alvei, volutin. These can be determined by the use of appropriate stains.

8. **Cultivation on such Substances as Sterilised Potatoes, Carrots, etc.** These are good as subsidiary cultivations.

After a species has been cultivated by all the methods detailed above, we have gained a large amount of information concerning it. But it must be remembered that we are dealing with organisms very sensitive to changes of environment and therefore liable to show different kinds of growths. So, for successful work, one must not rely on any one or two modes of cultivation, but must do as many as possible, and thus lessen the likelihood of confusing one organism with another.

	Source of Nitrogen.	Source of Carbon.	Chem. reaction.	Bac. anthracis.	Vibris cholerae.	Bac. subtilis.	Bac. pyocyaneus.	Bac. typhi.	Bac. coli.
1.	1 peptone	1 grape sugar	alkaline	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
2.	1 peptone	1 peptone	alkaline	+ +	+ +	+	+ + +	+ +	+ +
3.	1 asparagin	1 grape sugar	alkaline	0	+ + +	+ + +	+ + +	+	+ + +
4.	1 asparagin	1 grape sugar	acid	0	0	+ +	+ + +	+?	+ + +
5.	1 asparagin	1 asparagin	alkaline	0	+ +	+	+	0	+ +
6.	1 asparagin	1 asparagin	acid	0	0	+	+	0	+
7.	1 ammonium tartrate	1 glycerine	alkaline	0	+	+ + +	+ + +	0	+ +
8.	1 ammonium tartrate	1 ammonium tartrate	alkaline	0	0	0	+?	0	+?
9.	1 ammonium chloride	1 glycerine	alkaline	0	+ +	+ +	+ +	0	+ + +
10.	1 ammonium chloride	1 glycerine	acid	0	0	+	+	0	+ + +
11.	1 pot. nitrate	1 grape sugar	alkaline	0	+?	+ +	+ + +	0	+
12.	1 pot. nitrate	1 glycerine	alkaline	0	0	0	+ + +	0	0

+ + + indicates strong growth.
+ + indicates medium growth.
+ indicates small growth.
0 indicates absence of growth.

CHAPTER IV.

ACTION OF EXTERNAL INFLUENCES ON BACTERIA.

§ 1. INFLUENCE OF TEMPERATURE.

The examination of the influence of temperature must always be preceded by an observation of the temperature at which the organism ordinarily thrives, for bacteria are in this like human beings, some are adapted to live in what, to other bacteria, would be a cold climate; on the other hand, other organisms are adapted to a tropical life, represented in this case by hot springs, etc., in which bacterial life is not wanting. Hence, for every species there will be a minimum, optimum and maximum temperature at which growth takes place. It is usual to have three standards of temperature, viz. room-temperature, varying from 18° C. to 22° C.; lower-temperature incubator, 30° C. to 33° C.; and higher-temperature incubator, 35° C. to 38° C. Pathogenic bacteria thrive best at the last named temperature, which is approximately that of the human body. On the other hand, soil-bacteria, water-bacteria and air-bacteria require a lower temperature. They grow well at room-temperature, but somewhat better and quicker at the medium range, viz. 30° C. to 33° C. Of course a large number of pathogenic bacteria are present in the soil, water, and air, but they are either in the resting condition or else not growing at or near their optimum range of temperature. Whilst the average thermal death-point of bacteria is about 55° C. there are some, termed *thermophilic*, which can stand much higher temperatures. With regard to low temperature, the first recorded experiments are those of Forster in 1887. He found that in commercial milk there were 1000 bacteria per 1 c.c., in drain water 2000 per c.c., in garden soil 140,000 per grain, and in street mud an innumerable quantity per grain, which were not only alive, but reproductive at 0° C. Further, Miquel found that a

sample of sea-water at 0° C. which contained, when freshly taken, 150 germs per c.c., had 520 after 24 hours, and 1750 after four days.

Although their power of reproduction does not extend much below 0° C., their power of remaining unharmed on exposure to very low temperatures is considerable. Pictet and Yung found that some bacteria could be kept at −70° C. for 108 hours, and at −130° C. for 20 hours, without losing their power of growth when afterwards transferred to a suitable medium. It was also found that tubercle bacillus can be exposed to the temperature of liquid air (−193° C.) for continuous periods, varying from 6 hours to 42 days, without its vitality being affected. MacFadyen and Rowland experimented with Proteus vulgaris, Bac. coli communis, and others, and found them unimpaired after exposure of 10 hours to the temperature of liquid hydrogen (−252° C.). It will thus be seen that exposure to low temperatures is quite useless for purposes of sterilisation. The question is always one of penetration of the membrane of bacteria, for it must not be imagined that the protoplasm itself can stand these temperatures, but rather that the cell-membrane is a very bad conductor of heat and thus prevents the heat contained in the protoplasm from being dissipated.

Bacteria are very much more sensitive to heat than to cold, because, probably, the expansion of the membrane by heat facilitates the entrance of the penetrating hot fluid. The average thermal death-point is about 55° C., hence when non-sporing bacteria are placed in boiling water (100° C.) they are killed almost instantaneously. The thermophilic bacteria, however, can stand higher temperatures. Bac. thermophilus is a type of this class. It can grow actively at 70° C., which being 15° C. above the normal thermal death-point, is fatal to animal cells and to protoplasm generally. Miquel describes this form as producing short rods about 1 μ in thickness at 50° C., the rods becoming longer as the temperature rises. At 70° C. the whole field is occupied by these long threads. Its minimum temperature is 42° C. and maximum about 72° C. This species is very frequent in sewage and in the alimentary canal of human beings and other mammals. It is a saprophyte so far as is known, as it feeds on dead organic matter.

Another heat-loving form is Bacterium phosphorescens, found in the West Indies, which ceases to live below 15° C. Again, other bacteria have been found in boiling springs, the temperature of which was 61° C., or 9° C. above the normal thermal death-point. Again, in the hot sulphur springs at Ilidze, near Sarajevo in Bosnia, two forms, called

by their discoverer Bacterium Ludwigi and Bacillus Ilidzensis capsulatus were found, which apparently could develop only when the temperature rose above 50° C. The range of temperature is therefore very variable. Some, like Bacterium Ludwigi and Bac. Ilidzensis capsulatus, begin at 50° C., others, isolated in garden soil, were found by Globig to have a range from 15° C. to 68° C. in which development could take place. One garden-soil species seemed to grow only when the temperature got near 60° C. The maximum temperature of growth yet observed is that given for Bac. thermophilus, viz. 72 C. For others the maxima range from 55° C. to this temperature.

The following table shows the length of time which is necessary to prevent the development of soil bacteria when placed in water at 100° C. and 80° C. respectively :

	100° C. Minutes.	80° C. Hours.
Spores Bac. tumescens,	4–5	5–5½
,, ,, cohaerens,	4·5–5	8–8½
,, ,, simplex,	3–4	2⅔–2⅚
,, ,, mycoides,	10	8–8½
,, ,, pumilis,	6–7	7–7½
,, ,, fusiformis,	3–4	9–9½
,, ,, carotarum,	4½–5½	6–6½
,, ,, Ellenbachensis,	1–2	7–7½
,, ,, graveolens,	7–10	9½–10
,, ,, subtilis,	150–180	45–70
,, ,, ruminatus,	1·75–2	—

Roughly speaking, it requires at least 60 times as long at 80° C. as at 100° C., so that in sterilising spores, care should be taken to see that the temperature does not fall below the boiling point. In the case of organisms which do not form spores, among which, fortunately, are found the bacilli of diphtheria, consumption, typhoid fever, and most other pathogenic species, the resistance to a temperature of 80° C. is not great. In the case of Sarcina ureae, the following results were obtained after 5 seconds, 10, 15, 20, and 30 seconds respectively at 80° C. :

5 +, 10 +, 15 +, 20 −, 30 −.

(+ indicates growth, and − absence of growth.)

The vegetative cells of the same species, when subjected to 100° C., gave the following results :

5 secs. +, 10 secs. +, 15 secs. +, 30 secs. −.

These results indicate that the vegetative cells of bacteria are killed equally as rapid at 80° C. as at 100 C. and that in each case an exposure of 15 seconds is sufficient to effect their destruction.

With regard to the resistance to heat of the spores of the Coccaceae, the author has obtained the following result with the spores of the Sarcinae ureae:

A. at 80° C.

1 hour +, 1¼ hours +, 1½ hours +, 1¾ hours +, 2 hours −.

B. at 100° C.

2 mins. +, 3 mins. +, 3½ mins. −, 4 mins. −, 5 mins. −, 6 mins. −.

Hence the spores at 100° C. and at 80° C. have a power of resistance equal to that of the less resistant kinds among the Bacteriaceae.

The amount of resistance to heat of any particular species is not always constant. If two cultures of the same species, derived from different sources, be examined, some variability in this respect is sure to be found. Also, just as a gardener may in course of time obtain a new variety by carefully selecting forms which show the required characteristics in a most pronounced form for breeding, so in the same way it is possible to "breed" cultures which can stand more heat than the normal cultures. There appears to be no doubt also that nature does the same by a process of natural selection, by selecting from species with more resistant membranes, so that in course of time a variety is produced which is more resistant to heat than the parent species from which it was derived. This is the more probable because bacteria are amongst the most plastic of organisms. Apart from this, even in the different cultures of the same species, all of which have been derived from the same source, the resistance to heat depends on the age of the spores, their dryness, and probably on other factors unknown as yet. This variability is probably connected with differences in the thickness and density of the outer spore coats. Older spores in a dried condition are the more resistant, younger spores in a moist condition being more easily killed.

§ 2. ELECTRICITY.

When a current of electricity is passed through a nutrient medium containing bacteria, the inimical effect may be twofold. In the first place products may be formed by the action of the current on the medium which are detrimental to bacterial life, and in the second place the current may affect the bacteria directly. The first effect is seen in

Cohn's experiment. He passed a current through a nutrient solution, and ascertained that when two or more battery cells were used for over 12 hours the medium would not support bacterial life. That the bacteria were not directly affected was proved by the fact that when they were inoculated afterwards into another medium, normal growth was produced. It was suggested that this method could be used in the purification of sewage, and tentative experiments have been made in this direction. The current is generated by a large dynamo machine, and into the water which is to be purified are dipped two large electrodes. The water flows between the electrodes and becomes subjected to the influence of the current. According to Fermi, a current of 0·5 – 1·0 ampère reduced the number of germs to between $\frac{1}{10}$ and $\frac{1}{100}$ of the original number. Experiments were made by Burci and Frascani to ascertain the effect of an electric current apart from the effect of changes in the medium. The bacteria were dried on a pad of glass wool at 37° C., and then dipped into a mercury trough. As mercury is a good conductor of electricity, a current could be passed through this liquid, and its effect on the bacteria observed by subsequently inoculating them into a nutrient medium to see whether they were still capable of germination. It was found that the direct effect of an electric current is to kill bacteria.

Still more conclusive results were obtained by the experiments of Spilker and Gottstein. The bacterial material was placed in a flask round which was coiled a wire. An induction current was then passed through the wire, when, of course the bacteria, being in the electric field, became subject to electric influences. The organism experimented upon was Micrococcus prodigiosus. It was found that this form was killed off when a current of 2·5 ampères was passed for 24 hours through 250 c.c. of the nutrient material containing the bacteria. Cocci as a class are less resistant than bacilli, so that a stronger current was necessary to kill off the latter, and, when they were in the form of spores, it was found impossible to destroy completely all the spores in a nutrient medium, although the number could be *diminished*. An electric treatment is, therefore, not sufficient to sterilise milk, and its expense would render the method impractical when a simpler and less expensive method, that of applying heat, effects the same result. The only application that has been made of electricity in connection with nutrient fluids is in the artificial maturing of wines and cognacs to produce certain flavours. This, however, does not depend upon bacterial effects, but upon the production of chemical changes which

influence the flavour of the beverage treated. Again, the effect of electricity has been tried on the chromogenic power of bacteria. Bac. pyocyaneus, which produced a blue pigment, was placed in the cavity of a solenoid, and subjected to a current of 10,000 volts. An exposure of twenty minutes almost completely destroyed the chromogenic power of this bacillus.

Magnetism. In all experiments in which bacteria have been placed in a magnetic field only negative results have been obtained. It must, however, be stated that the question has not been sufficiently investigated.

§ 3. LIGHT.

The importance of light as a germicidal agent is an important point to note in the cultivation of bacteria, as the vast majority not only thrive better in the dark, but if exposed to the light, and especially to strong sunlight, are very soon killed off. This was proved as early as 1877, when it was found that the direct rays of the sun were prejudicial to the growth of bacteria. Diffuse daylight is also inimical, though less so than the direct rays. At this time it was also proved that the blue and violet rays of the spectrum were the most active destroyers, and next to them the red and orange-red rays. The question of the germicidal power of the various rays of the spectrum has recently been again investigated, and it is now known that from red to green the effect is almost nil, from red to the violet end of the blue the effect increases, reaching its maximum at the latter point, and beyond it in the ultra-violet rays there is a falling off in effect. The rays of the electric light are also germicidal, but less so than those of direct sunlight. The effect of light seems to be independent of temperature, for Tyndall found that even on the Alps the growth of organisms which he had taken up enclosed in a flask, was influenced by exposure to sunlight.

As to the exact method by which the light destroys bacteria all observers are not agreed, but it is held by some that light produces decomposition products in the nutrient media, which act inimically on the bacteria. By others it is held that light acts directly on the organisms apart from its effect on the surrounding medium. It is, nowadays, generally supposed that in nature both factors are instrumental in accomplishing the same end; thus light acting on urine produces hydrogen peroxide, which is an antiseptic, and therefore kills off the bacteria that are feeding on the urine. In the majority

of cases the effect of this decomposition is such that the food is rendered unsuitable for the bacteria that happen to be present, though it is not necessarily poisonous. The bacteria therefore die of starvation and not from the effects of poison.

The maximum effect of light is attained in large, shallow pools, where the water is stationary or moving only very slowly. Buchner found that the effect of light was bactericidal after penetrating 15-20 inches of water, though it must be stated that another investigator, Arloing, holds that sunlight cannot be bactericidal to a depth of even one inch. This contradictory evidence is possibly to be explained by the fact that one may have worked with spore-material and the other with vegetative cells, for the spores, of the soil-bacteria, for example, may be exposed to the sun for weeks, possibly years, without losing their vitality, whilst vegetative cells are much more susceptible. Hence, in examining the effect of light on material contained in water, we must first examine the nature of that material to ascertain whether spores are present. A familiar experiment demonstrating the effect of light, is to sow Bac. typhosus on nutrient gelatine in a Petri-dish, and after covering up a portion of the surface with black paper, to expose the plate to the light. Growth will take place only under the black paper. The same can be performed in an agar-tube by covering the lower portion of the tube with black paper, but leaving a small portion uncovered. Growth will not take place where the light reaches the the agar.

Lately the effect of light on Bac. typhosus has been carefully examined by the State Board of Health of Massachusetts. The table shows the elimination of this organism in water on exposure to direct sunlight. It shows that 15 minutes' exposure to direct sunlight has a very powerful effect on this bacillus, as the number is reduced to 14 per cent. of the original number.

Exposure.	No. of Bacteria (Average of Experiments).	No. of Bacteria (Average of another set of Experiments).
Start	716	562
15 minutes	35	9
30 minutes	9	4
45 minutes	1	2
1 hour	4	13
1½ hours	1	4
2 hours	0	4
4 hours	2	0
6 hours	0	0

Bac. typhosus, however, is not a highly resistant form; but even Bac. coli communis was reduced to 20 per cent. of the original number after 15 minutes' exposure to direct sunlight, and all the organisms were destroyed after 4 hours' exposure. These two organisms, however, do not form spores. Ward has tried the effect of direct sunlight on spores of Bac. anthracis and stated that two to six hours' exposure produced a germicidal effect.

The effect of light on pigment-producing bacteria must here be noticed. It sometimes happens that these bacteria when cultivated for some time lose their power of producing pigment, which power, however, may unexpectedly return. The conditions affecting this loss of colour are unknown in most cases, but it is known that Bac. lactis erythrogenes invariably loses its power of forming a red colouring matter when strongly illuminated. Again, in the case of the phosphorescent Photobacterium sarcophilum, the power of producing phosphorescence is lost when the organism is allowed to grow in a lighted room.

While the vast majority of bacteria are thus disastrously affected by light, the purple-bacteria always grow in places exposed to strong sunlight, and it has been claimed that they are partially dependent upon light for their food supply. These bacteria will be dealt with in a later chapter.

§ 4. MOISTURE.

Like all living organisms, bacteria require moisture, the amount required varying with different species. For example, Spirillum giganteum will not grow on a very dry medium, and the same is probably true of all spirilla, but Bac. subtilis, and most of the soil-bacteria grow well on very dry nutrient media. Consequently, when drought occurs, the spirilla perish first, and are followed by the vegetative cells of all genera. The spores are naturally the most resistant, for their coats do not permit the water of the enclosed protoplasm to pass through for a very long time, and they have been known to be capable of germination after having been in a dried-up condition for many years. The importance of water will be readily seen when it is considered that more than three-fourths of protoplasm consists of this liquid.

§ 5. MECHANICAL AGITATION.

It was at one time thought that no living organism could continue to exist unless it obtained a certain amount of repose for reproductive purposes. It was, however, demonstrated that there were algae living beneath large waterfalls, and consequently always subjected to agitation, which were evidently able to exist and to carry on their reproductive processes even whilst being violently agitated. However, bacteria do suffer if violently agitated. Thus, it has been found that when they were placed in a shaking machine which made a hundred movements per minute, each with an amplitude of ten inches, for 48 hours, the agitation proved fatal to the bacteria. Conclusive results were obtained by Meltzer. He used the agitator of a New York mineral water works, which subjected the samples to 180 reversed movements per minute, the amplitude of each movement being 40 cms. This process decreased the number of germs, and, when it was long continued, they disappeared altogether. When glass beads were added to the sample, the experimenter found that the process of extinction was expedited, ten hours being sufficient to kill the whole number present. He also found that there is a difference in the degree of resistance offered by bacteria, some holding out longer than others. As a result of the shaking the bacteria were split up into an extremely fine powder. The same observer found that when cultures of Bac. subtilis or Bac. megatherium were placed for four days in the engine room of a brewery, where, in consequence of the working of the engine, the whole room was subjected to vibration, all the bacteria in the cultures were destroyed.

When not too violent, however, motion is favourable to the well-being of bacteria and allied organisms. Thus, E. Chr. Hansen found that yeast developed better when the beer-wort was set in motion by stirrers. In the case of Bac. ruber it has been ascertained that the power of reproduction is improved by slight movement. When, starting from rest, the motion is gradually increased, an optimum is reached, beyond which the motion impedes the power of reproduction until finally a point is reached when the motility is such as to cause destruction. As would be expected, the optimum varies for different species, the hardier bacilli, for example, being able to stand a good deal more shaking than the weaker spirilla.

§ 6. GRAVITY.

The earth exerts an attraction for bacteria as for all other substances. The effect of this influence is indicated by the number of bacteria at different altitudes, the number nearer the ground being greater than at a higher level. This can be demonstrated by the following tables:

Top of Primrose Hill,	- -	9 organisms per litre.
Bottom ,, ,,	- -	24 ,, ,,
Norwich Cathedral—		
Spire (310 feet high),	-	7 bacteria per 10 litres.
Tower (180 feet high),	-	9 ,, ,,
Ground,	- - - -	18 ,, ,,
St. Paul's Cathedral—		
Level of Golden Gallery,	-	11 ,, ,,
,, Stone ,,	-	34 ,, ,,
Churchyard,	- - -	70 ,, ,,

It is therefore seen that the nearer we are to the ground the greater is the number of microbes, and that at high altitudes there are practically none at all, except such as are occasionally carried up by air currents. At the top of Mont Blanc the examination of 100 litres of air did not reveal the presence of a single microbe, and the total number of organisms, including bacteria and moulds, was found to be from four to eleven per 1000 litres.

CHAPTER V.

§ 1. MOTILITY IN RESPONSE TO EXTERNAL STIMULI.

We have seen that bacteria are motile, and we have now to consider in what manner external factors affect the power of movement. The most important is the effect which is known as *Chemiotaxis*, the name given to the power of attraction or repulsion which certain chemical substances possess, for motile organisms. The best known example is the attraction of oxygen for motile aerobic bacteria. If an aerobic bacillus be placed in a drop of water and a glass coverslip be placed over the latter, the individuals will swim towards the edges, being attracted by the oxygen of the atmosphere. Conversely, an anaerobic bacillus will be repelled by the oxygen of the atmosphere, and, under the same circumstances, will move towards the centre of the coverslip because this point is furthest removed from the oxygen. In these cases the reason for the respective movements is obvious, for the well-being of these bacteria depends in the one case on the presence of oxygen, in the other on its absence. But attraction or repulsion is not always guided by this consideration, for corrosive sublimate, the most powerful germicide we know of, attracts bacteria, whilst most of the substances which are used for the nutrition of bacteria are quite neutral in this respect.

Pfeffer has given us a good method of ascertaining the chemiotactic power of any particular substance. A small portion of a fine capillary tube, closed at one end, is first filled with the liquid to be examined, and then placed in a drop of liquid containing some motile microbe. The fineness of the bore prevents the liquid from running out of the tube. If this liquid has an attraction for the bacteria, it will be found that there is a concentration of the latter round the entrance to the tube. On the other hand, if the liquid be neutral, no change in the distribution of the bacteria will be observed, whereas if it exerts

repulsion the bacteria will recede from the capillary tube as far as possible. By this method the attraction or repulsion for bacteria of various substances has been investigated. In general it may be stated that all metallic salts exert attraction, the most powerful being those of potassium, sodium, rubidium, calcium, barium, and strontium. Thus a 2 per cent. solution of common salt (sodium chloride) exerts a powerful attraction. On the other hand, all salts with an acid reaction repel bacteria, and the same may be said of all free acids, free alkalies, and alcohol. Among organic substances, peptone and asparagin in weak solutions are strongly positive, carbohydrates, *e.g.* sugar, are weakly positive, whilst glycerine is neutral. A very essential point to notice is that a substance which is positive in a weak solution may act as a repellent in a stronger solution, this being the case with peptone and asparagin for example. Whilst a 2 per cent. solution of common salt is positive, 19 per cent. and stronger solutions are entirely negative. The same holds for most of the other substances that are positive in their action.

A peculiar fact in connection with their movements is, that bacteria are subject to *Weber's Law*. To explain this law, suppose that a weight of 1 lb. be placed on the hand. It has been found that it requires the addition of $\frac{1}{3}$ lb. before the hand *feels* that an additional weight has been placed on it. If 2 lbs. be placed on the hand instead of 1 lb., it will now be found that $\frac{1}{3}$ of 2 lbs., that is $\frac{2}{3}$ lb., will have to be added before the hand feels that an additional weight has been placed on it. That is to say, the weight that must be added before an addition is felt, is always $\frac{1}{3}$ *of what is already on the hand*. This is expressed by saying that the *perception-coefficient is* $\frac{1}{3}$. That bacteria obey this law may be seen from the following experiment. A small piece of a capillary tube is filled with a 1 per cent. salt solution in which some motile bacteria are contained, and another tube with a $\frac{1}{2}$ per cent. salt solution containing the same bacteria. If, now, both tubes be placed in a drop of a 3 per cent. salt solution, the latter exerts a chemiotactic attraction for the bacteria in the tubes. But whilst the bacteria in the tube containing the $\frac{1}{2}$ per cent. salt solution will move out of the tube, those in the other tube will not do so. The reason of this is that the *perception-coefficient* for bacteria is 5, hence, to ensure movement, the percentage of salt in the drop must be at least 5 times that of the salt in the tube. The salt in the drop is 6 times more concentrated in the case of the one in which movement of the bacteria takes place, but only 3 times more concentrated in the case of the other, so that movement does not take place.

§ 2. RESPIRATION OF BACTERIA.

All organisms, animal and vegetable, must respire. To understand what we mean by this, let us consider what happens when we ourselves respire. The protoplasm of our bodies is constantly being broken down into simpler substances, the process resulting in the liberation of a certain amount of energy, which we employ in a variety of ways, as in the warming of our bodies, the repairing of waste tissues, in short, respiration supplies the energy for the continuation of the vital functions. The same applies to bacteria, the vast majority of which take up oxygen as we do, in order to effect the decomposition of protoplasm. Without this absorption of oxygen neither ourselves nor the bacteria (with the exception of a few, the anaerobic bacteria) are able to effect this decomposition.

Respiration results in the formation of a large number of substances, as the decomposition of protoplasm does not take place in one but in a series of steps. Of the various substances formed, some are useless and are got rid of. These are the *excretion products*. Thus in our own case, the carbon dioxide that we exhale from our lungs is such a product. Many of the bacterial excretion products have been identified and some have already been mentioned in a previous chapter. Other substances formed by the decomposition of protoplasm are utilised once more in the building up of protoplasm, which must naturally take place to make up for the loss due to decomposition, and for purposes of growth and multiplication. It will readily be seen that all the general principles which underlie the physiology of the higher animals and plants apply also to bacteria, for like all other organisms, these minute plants are composed of protoplasm, which is essentially the same throughout the whole of the animal and vegetable worlds.

§ 3. THE FOOD OF BACTERIA.

In discussing the food of bacteria, it is well to remember that the essential ingredients depend on conditions similar to those which prevail in the case of higher plants and of animals. We must provide those substances which the organism needs for the building up of its own body, and in addition, a number of substances that are not constituents themselves, but which help in the building up of these

constituents. In green plants, for example, a very small quantity of iron is necessary; because, without it no chlorophyll can be formed, and without chlorophyll there can be no building up of carbon dioxide and water into formaldehyde, which is the first step in the construction of organic substance. To find the nature of the substances that enter into the composition of any organic body a chemical analysis must be made. An analysis of this kind for bacteria shows that they do not essentially differ from other organisms. The elements present are carbon, hydrogen, oxygen, nitrogen, sulphur, phosphorus, potassium, calcium, iron, and magnesium. In addition, for many, especially pathogenic bacteria, sodium, and chlorine, though not absolutely necessary, yet make a great difference to the well-being of the organism. In the list just given the first six are necessary, because protoplasm consists of these elements. The others mentioned are found to be necessary because, just as in other plants, they are connected with the machinery, as it were, by which protoplasm is constructed. Thus calcium is necessary probably to neutralise the poisonous effect on protoplasm of acids secreted as waste products. With regard to iron, all green plants and almost all the lower animals require this substance, though in such small quantities that no special provision need be made for its presence. The same is true for bacteria, but the exact rôle of these elements is not yet ascertained. To supply the necessary mineral constituents the following mixture may be used in making up nutrient solutions:

($CaCl_2$) Calcium chloride,	0·1 gram.
($MgSO_4$) Magnesium sulphate,	0·2 ,,
(K_2HPO_4) Potassium hydrogen phosphate,	0·1 ,,
Distilled water,	1000 c.c.

In ordinary cultures, however, where the exact analysis of the nutrient solution is not required, ordinary tap-water may be used instead of this mixture, especially if flesh extracts are used in the preparation of the nutrient media. It will be noticed that the proportion of water is very great, but it must be remembered that the proportion of water in living protoplasm is very high, that no food can be taken except in solution in water, and also that the process of digestion, that is the changing of insoluble into soluble substances fit for absorption, cannot take place unless water be present, for ferments act only in the presence of water. Another reason for this large percentage lies in the fact that if the nutrient substances

are too concentrated, they tend to withdraw water from the cells, with disastrous results. It is, therefore, always preferable to risk making the solutions too dilute than too concentrated. Again, a solution which, when dilute, may be a nutrient material, is often an antiseptic in the concentrated form. An example of this is sugar, which up to 10 per cent. can serve as food to bacteria, at 50 per cent. is a strong antiseptic.

Bacteria have been known to thrive in a liquid containing only sugar. In fact, it was stated by Fermi that he had obtained bacteria which had no nitrogen in their composition, because he claimed to have cultivated them in a solution of water and sugar. It has since been shown that Fermi's solution had in it very small quantities of foreign matter derived from various sources, and these quantities, though extremely small, were yet sufficient to supply the nitrogen required. This shows that bacteria can thrive in extremely dilute solutions.

Of the various elements necessary for the building up of the bacterial cell oxygen and hydrogen are amply supplied by the water. They are also supplied by the various compounds which are used as food-material for bacteria, and into whose composition they enter largely. With the exception of two, carbon and nitrogen, we may assert that the same applies to all the other necessary elements.

With regard to carbon, this substance is necessary, because it forms a large proportion of the body of the cell. Also, the energy which the bacteria require to perform the various functions of life, division, multiplication, growth, etc., is best obtained by the breaking down of some carbonaceous compound like sugar, or a similar carbohydrate. In making our medium we must, therefore, for most purposes, make provision for this supply. In most of the nutrient media that are used for the cultivation of bacteria, carbon is supplied in the form of flesh-extract, peptone, sugar, etc. The necessary energy can, however, be supplied in other ways than by the breaking down of carbonaceous organic material. For instance, the sulphur-bacteria derive additional energy by the oxidation of sulphuretted hydrogen, first into sulphur, and later into a sulphate. It, therefore, follows that we can cultivate these bacteria with a very small quantity of carbonaceous material. Another class, the nitrate-bacteria, which transform nitrites into nitrates, can be cultivated without having carbon supplied to them in the organic form, in fact, they seem rather to suffer when such material is presented to them.

A solution commonly used for the cultivation of the nitrate-bacteria is one composed of the following:

Sodium nitrate,	1 gram.
Sodium carbonate,	1 ,,
Potassium hydrogen phosphate,	0·5 ,,
Common salt,	0·5 ,,
Ferrous sulphate,	0·4 ,,
Magnesium sulphate,	0·3 ,,
Water,	1000 c.c.

The carbon for the building of the bacterial cell is thus derived entirely from the inorganic sodium carbonate (Na_2CO_3) and from the carbon dioxide of the atmosphere. The same applies to the nitrite-bacteria which change ammonium compounds, formed by the putrefaction of organic remains, into nitrites. The composition of the solution commonly used in the cultivation of nitrite-bacteria is the following:

Ammonium sulphate,	2 grams.
Magnesium sulphate,	0·5 ,,
Potassium hydrogen phosphate,	1·0 ,,
Common salt,	2·0 ,,
Ferrous sulphate,	0·4 ,,
Distilled water,	1000 c.c.

In this case the carbon necessary for the construction of the body of the cell is derived entirely from the carbon dioxide of the atmosphere. The nitrite- and nitrate-bacteria thus resemble the higher green plants, which also get their carbon dioxide from the atmosphere, but there is this difference: the higher green plants require the green colouring matter, chlorophyll, and also the energy of the sun's rays, before they can accomplish the assimilation of carbon dioxide, whereas these bacteria can do this without their aid.

It has been claimed that another class of microbes, the *purple-bacteria*, obtain their carbon supply in the same way as green plants. These possess a colouring matter called *purpurin*, which has been stated to play the same rôle as the chlorophyll of green plants. If this be true, they obtain energy in two ways. First, they obtain it by assimilation, whereby more complicated compounds have been elaborated from the carbon dioxide of the atmosphere, for these compounds, when broken down again, will supply energy. Secondly, they obtain it in virtue of the fact that they are also sulphur-bacteria,

therefore the oxidation of sulphuretted hydrogen to sulphates also takes place, thus liberating another supply of energy.

Now we must deal with the modes in which the carbon must be supplied to the bacteria. The best are the various forms of sugar, peptone, fats, and proteids. It has also been obtained by the addition of alcohol to the nutrient media. Thus glycerine (which is one of the alcohols) is a food-stuff to some bacteria, whilst the acetic-acid bacteria can derive their carbonaceous supply by the decomposition of ethyl-alcohol. Some of the fatty acids, *e.g.* formic and acetic acids, may be employed in the cultivation of some bacteria, but their food value is not great. In some combinations the carbon is quite useless to the bacteria. Examples are the cyanide compounds, salts of oxalic acid and urea. Of course all the compounds of carbon which are in any way antiseptic are useless as food materials.

Let us turn now to the nitrogen supply. As in the case of carbon, provision must be made for this, but great care must be taken in the nature of the supply, for the different kinds of bacteria require the nitrogen in particular forms, otherwise they cannot use it. For the cultivation of the majority of forms nitrogen is supplied by means of flesh extracts, proteids, peptone, serum, etc. In the case of the nitrite-bacteria, the nitrogen-supply must be an ammonium compound. The nitrate-bacteria must obtain their nitrogen in the form of a nitrite. Again, still another class obtain their nitrogen direct from the free nitrogen of the atmosphere. These different kinds are discussed more fully in later chapters.

§ 4. CHIEF CONDITIONS REGULATING GROWTH.

In considering the growth of bacteria, we must treat them in the same way as we treat all living organisms, for not only must we supply food but also water, a suitable temperature, and, in the case of the majority of bacteria, free oxygen. It has already been stated that more than three-fourths of the protoplasm of all organic bodies consists of water. An animal deprived of water and given plenty of solid food succumbs much sooner than if supplied with plenty of water but with no solid food. The same applies to bacteria. In making up nutrient media, the amount of solid material in comparison to the amount of water is very small, in fact, some bacteria seem to be able to find enough food in distilled water. This question of the proportion of solid food is extremely important for the reason already mentioned,

namely, that there are some substances which, in a concentrated solution, are able to abstract water out of the protoplasm of bacteria, thereby causing their death.

Suppose we place in a bucket of water a bottle full of alcohol, closed at the top with a piece of parchment. After a time it will be found that the parchment bulges outwards, showing that some water has got into the bottle. It will also be found that some alcohol has gone out into the water, and the bulging of the parchment is an expression of the fact that less alcohol has gone out than water has passed into the bottle. If we had placed a bottle of water, with a similar parchment covering, in a bucket of alcohol, the parchment would be found to bulge inwards. This interchange of liquids through a permeable membrane is called *Osmosis*, and plays an important rôle in the absorption of food material in the case of plants. In the case of bacteria, the protoplasm represents the alcohol, the membrane the parchment, and the nutrient medium the water of our illustration. Under favourable circumstances of growth the protoplasm sucks in the nutrient medium at a far greater rate than the latter sucks out the water of the protoplasm, for the protoplasm itself cannot bodily pass out. If, however, the concentration of the nutrient solution be increased, the preponderance is reversed, and more water passes out than nutrient solution passes in. This causes the protoplasm, deprived of its water, to contract, when it is said to be *plasmolysed* (Fig. 64). Unless this is speedily remedied, a plasmolysed cell is soon rendered incapable of growth, and death ensues. Hence, in making a nutrient solution, care must be taken to prevent the food material being too concentrated, in case some of its compounds may have this plasmolysing power. Thus, 5-10 per cent. cane sugar is a good nutrient material, but a 50 per cent. solution acts as an antiseptic. The high concentration of the sugar in many jams is probably the cause of the comparative immunity from the attacks of moulds which many jams and similar articles enjoy. Again, common salt is used as a food material, but if it is present in a concentrated solution bacterial growth is impossible.

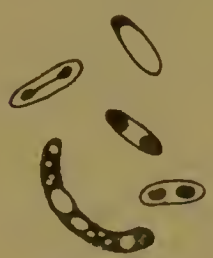


Fig. 64.—Plasmolysed cells.

In considering the relation of water to bacterial life, we must also bear in mind that absorption of food can be effected only when the latter is in a state of solution, as no solids or gases can pass through the membranes of bacteria.

Now many solids which bacteria use as food, for example, proteids, are not soluble in water, so, in the form in which they are presented, they are useless. To overcome this difficulty, bacteria, like other living organisms, have the power of secreting what are called *ferments* or *enzymes*, the chief function of which is to change the insoluble into soluble substances, which can then pass through the membrane. To effect these transformations water must be taken up by the ferments, otherwise they are not able to operate successfully, so that this is another reason for the necessity of the presence of water in the culture of bacteria.

Next, with regard to oxygen, up to 1861 it was thought that without oxygen no organic life was possible. In that year, however, Pasteur discovered some bacteria which were able to thrive in an environment devoid of oxygen, and since then several similar bacteria have been discovered. With these exceptions, however, oxygen is essential to the life and growth of bacteria.

Finally, we have to consider the necessity of an adequate temperature. As already explained, bacteria are not uniform in this respect: some, by gradual acclimatisation to hot springs, are rendered capable of active multiplication at a temperature which would be almost instantly fatal to others. Also, owing to extreme sensitiveness to change of environment, the minimum, optimum, and maximum temperature will vary with the species and with the different cultures of the same species. High temperatures produce more disastrous and immediate effects than low temperatures. At 0° C. there is no multiplication: the optimum for most bacteria lies between 30° C. and 40° C., whilst, with a few exceptions, 45° C. is the higher limit. The optimum temperature for almost the whole of the non-pathogenic bacteria lies very near 32° C.

The following table gives the minima, optima, and maxima of a few of the pathogenic bacteria:

	Min.	Opt.	Max.
Bac. subtilis, - -	6°	30°	50°
Microspira comma, -	8°–12°	37°	40°
Bac. anthracis, - -	12°	37°	43°–45°
Bac. diphtheriae, - -	18°–20°	33°–37°	40°
Bac. influenzae, - -	26°–27°	37°	43°
Bac. tuberculosis, -	29°	37°–38°	41°

(Degrees—Centigrade).

§ 5. PHYSIOLOGICAL CLASSIFICATION.

We may divide bacteria according to their mode of life, for there is a great deal of difference between the various kinds of bacteria in this respect. Thus, some feed on living organic-, others on dead organic-matter, whilst to a third kind, the presence of organic matter exercises an injurious influence on its growth. Others, again, are characterised by the possession of special properties, *e.g.* the power of exhibiting phosphorescence, of secreting colouring matters, and so on. In a classification of this nature, it does not follow that the inclusion of an organism in one group means that it is excluded from any of the others. For descriptive purposes such a classification is extremely useful. On a physiological basis we may divide the bacteria into twelve groups:

I. **Pathogenic Bacteria.** This term is applied to bacteria that feed on *living* organic matter and producing injurious effects; thus the bacilli causing diphtheria, consumption, lock-jaw, and other diseases, are pathogenic. Also bacteria attack wounded plants, often causing their death.

II. **Saprophytic Bacteria.** Applied to such as feed on *dead* organic matter. When an animal or a plant dies, it becomes food for a host of bacteria and other organisms, which are already present on its surface, or which drop down from the atmosphere. These effect changes which render the body a suitable feeding material for other bacteria with different physiological properties.

III. **Chromogenic Bacteria.** The term is used to designate those bacteria, the growths of which, on the various nutritive media, show distinct coloration. Thus, *Sarcina ventricula* shows a yellow, and *Micrococcus prodigiosus* a red growth. Almost all the colours are represented; they are extremely useful as guides in the identification of species.

IV. **Zymogenic Bacteria.** These are bacteria that induce fermentative changes. The use of the term zymogenic is somewhat ambiguous, because, as will be explained later, there is no general agreement as to what constitutes a fermentative change. Speaking generally, it is customary to apply the term zymogenic to those bacteria, small amounts of which produce a very large amount of chemical change in food and other substances, into which they have been inoculated. Further, the activities of these bacteria, in so far as these changes are concerned, are inseparable from their life-processes. A typical

zymogenic change is the souring of milk through the activity of lactic acid bacteria.

V. **Photogenic Bacteria.** The growth of these bacteria is attended by the production of phosphorescent light. The phosphorescence sometimes seen in a piece of decaying fish or of flesh is caused by the growth of these bacteria.

VI. **Thermogenic Bacteria.** Applied to those bacteria which, in their growth, raise the temperature of the medium in which they are growing to an appreciable extent.

VII. **Nitrite- and Nitrate-Bacteria.** These bacteria are peculiar in requiring only inorganic substances for their growth and multiplication. The nitrite-bacteria cannot thrive unless some ammonium-compound is the source of their nitrogen supply. The conditions of growth of the nitrate-bacteria are almost identical, the chief point of difference being the fact that they require a nitrite- and not an ammonium-compound to supply them with the necessary nitrogen.

VIII. **Nitrogen Bacteria.** These are characterised by their power of taking in or "*fixing*," as it is called, the free nitrogen of the atmosphere. During the decomposition of organic matter, a certain amount of nitrogen in the free condition is liberated into the atmosphere. Accumulation of this gas is stopped, however, by the activities of these bacteria, which bring the nitrogen back again into the soil.

IX. **Denitrifying Bacteria.** These organisms change nitrates and nitrites into free nitrogen, which then escapes into the atmosphere as a gas.

X. **Sulphur Bacteria.** The peculiarity of these bacteria is their power of absorbing sulphuretted hydrogen (H_2S), which, after absorption, is oxidised with liberation of sulphur. Microscopically examined, the bodies of these organisms are seen to contain bright globules of sulphur. Hence the name of sulphur bacteria. The sulphur is further oxidised into the sulphate.

XI. **Iron Bacteria.** These bacteria thrive in water containing iron, chiefly in the form of ferric hydroxide. The redness of what is known as iron-water is due, in almost every instance, to the presence of vast numbers of iron-bacteria, on the membranes of which an accumulation of ferric hydroxide has taken place. There is no general agreement as to the physiological process resulting in the deposition of this iron-compound on the membranes of these bacteria.

XII. **Purple Bacteria.** Finally, we must mention the purple bacteria, all of which are also members of the sulphur bacteria. In addition to possessing the characteristics of the sulphur bacteria, they possess also

the power of utilising their colouring matter in the same way as the green plants make use of their chlorophyll, viz. to absorb carbon dioxide from the atmosphere, making use of the energy of the sun's rays for this purpose. The name bacterio-purpurin has been given to the colouring matter of the purple bacteria.

In later chapters, these groups of bacteria will be dealt with in greater detail.

CHAPTER VI.

DISTRIBUTION OF BACTERIA.

§ 1. BACTERIA IN THE AIR.

As there is nothing in the air which can serve as food for bacteria, the number of these organisms in the atmosphere of any particular place depends altogether on the underlying surface. If the constitution of the latter is of such a nature that it cannot support bacterial life, or if the bacteria that are in it cannot escape into the atmosphere, then we find the atmosphere altogether devoid of bacteria. Thus it has been found that at the top of Mont Blanc there are only 4-11 bacteria in 1000 litres of the atmosphere, that there are any at all being due to the fact that a few are periodically blown up from the valleys. In places like the Arctic and Antarctic regions, and in the middle of great oceans and of great deserts, the atmosphere contains no bacteria. On the other hand the greatest number of bacteria will be found in the atmosphere of those places where conditions favour bacterial activity in the underlying surface, and also where the conditions are such that it is possible for the bacteria to escape into the atmosphere. The air of sewers is singularly free from bacteria, even although sewage contains many millions of bacteria per cubic inch, because of the moistness of the surfaces, within which the sewage is enclosed. The greatest number of bacteria is found in the atmosphere of large towns, and especially in those places where a large number of people are congregated in a small space and under insanitary conditions.

On the whole, the average number of bacteria in the atmosphere is very small, and 100 per cubic metre has been accepted as a fair average. It is thus estimated that a person who has lived up to the age of 70, has inhaled about 25,000,000 bacteria. A number far

larger than this enters the body every time half a pint of fresh milk is swallowed.[1]

The following statistics give a very fair idea of the distribution of bacteria in various places:

	No. per Cub. Metre.
Mont Souris Park (Paris),	455
Middle of Paris,	4,000
Tailors' Room, Whitechapel,	17,000
Capmakers' Room,	9,000
Boot Workshop,	25,000
Railway Works, Wilts,	20,000
Chocolate Factory,	8,000
Printing Shop,	9,000
Ropemakers' Shop,	20,000
Terrace of House of Commons,	4,200

The bacteriological examination of the atmosphere is best carried out by means of the Sedgwick sugar tube. This tube is about a foot long, of which half has a bore of 2·5 cm., while the other half has one of ·5 cm. After sterilisation an inch or more of the narrow part is filled with sterilised granulated cane sugar. A sterilised india-rubber tube is then attached by one end to the narrow part, and by the other end to an aspirator. A measured quantity of air is drawn through (about 10 litres), and of course all the bacteria are caught in the sugar. Then warm nutrient gelatine is poured into the broad part of the tube, and into this the sugar is thrust by means of a sterilised glass. After the sugar has melted the tube is turned round so as to spread the nutrient gelatine over as large a surface as possible on the glass. When it has solidified the tube is set aside to let each microbe that was inhaled by the tube grow into a colony. By counting the colonies we can estimate the number of bacteria that have been inhaled. As we know the volume of air passed through the sugar we can estimate the number of bacteria per unit volume.

Conditions determining the Bacterial Contamination of Air. Speaking generally, we may say that the more dust in the atmosphere the greater the number of contained microbes, because dust particles are usually covered with them. That such is the case can be proved by making a bacteriological examination of the air in a room, first when the dust is undisturbed, and then after it has been raised, when a considerable

[1] Lest a false inference be made from this assertion it is well to observe that whereas the swallowed germs enter the stomach, those that are inhaled enter the lungs and air passages which do not offer anything like the same resistance to the attacks of pathogenic bacteria.

difference will be observed in the two results. This was tried in a classroom of the Dundee High School. A bacteriological examination was made before and after the boys had been asked to stamp on the floor. Before stamping, 11 bacteria, and after, 150 bacteria were found per litre. We may, therefore, conclude that the atmosphere of towns contains more bacteria than that of the country, that we inhale more bacteria when on the highroad than when in the green fields, and more when walking through a fog than when walking through a clear atmosphere. The number of bacteria, however, does not always bear a relation to the amount of dust in the atmosphere. Thus, the air in the workroom of a certain skin-curer was found to be densely impregnated with dust particles from the skins, but there was scarcely a microbe present, while, on the other hand, the atmosphere of the polishing-room of a hat firm was found to be remarkably free from dust, yet several kinds of bacteria were isolated.

Secondly, the number depends on the dampness of the surfaces. The scarcity of bacteria in sewers has already been mentioned. The same condition explains the fact that during quiet breathing no bacteria are exhaled from the mouth, because the mouth and air passages being moist, the bacteria are all captured. During speaking, sneezing, and coughing, however, the case is different, for minute particles containing bacteria are shot out of the mouth during these processes.

Thirdly, the altitude of any particular place is an important factor, for, as bacteria are subject to gravity, the higher up we go the fewer bacteria do we find. This is clearly seen in the results of the investigation into the condition of the atmosphere of the House of Commons. At the top of the Clock Tower there were only 1·3 bacteria and moulds per litre, while halfway up this tower the number was 1·5. Twenty feet from the ground the number rose to 3·3 per litre, and on the ground itself 4·2 per litre were found. The same results have been obtained in other investigations of this nature. Thus, on the spire of Norwich Cathedral (310 feet) 10 litres of air yielded 7 micro organisms, whereas on the tower (180 feet) 9 were obtained, and on the ground 18 for the same quantity of air.

Fourthly, as bacteria are usually conveyed on particles of dust, we have to take into account the influence of air-currents on the distribution of bacteria. Other things being equal, there are more bacteria in the atmosphere during a high wind than when the weather is calm, owing to the amount of dust that the wind lifts from the ground.

Finally, the specific nature of the soil which creates the dust exercises a marked influence on the number of bacteria in the overlying

atmosphere : the greater the number of bacteria in the soil the greater the number that are found in the atmosphere. It therefore follows that the air above soils containing much organic matter, *e.g.* a tract of cultivated ground, contains more bacteria than the atmosphere above, for example, a sandy waste which has little or no organic matter in its constitution.

§ 2. BACTERIA IN WATER.

The number of bacteria in any liquid depends entirely on the capacity of the latter to sustain bacterial life. Bacteria are entirely aquatic in their habits, for even when growing on an apparently solid medium, *e.g.* nutrient agar, each individual is covered with a film of water which completely surrounds it. If, therefore, a liquid contains all the elements necessary for bacterial life, and other conditions are favourable, the number of bacteria becomes enormous. In milk and sewage we have examples of such liquids. The following table shows how numerous bacteria are in sewage water.

Crude Sewage.	No. of Bacteria per c.c.
1. Chiefly domestic sewage,	14,240,000
2. Mixed sewage,	7,800,000
3. Chiefly domestic sewage,	4,800,000
4. Mixed sewage and trade effluent,	36,000,000
5. Hospital sewage,	2,800,000
6. Domestic sewage and trade effluent,	4,100,000
7. Domestic sewage,	28,100,000
8. Mixed sewage,	21,100,000

Analyses of samples of milk, which have been allowed to stand for some hours, also show enormous numbers. In the case of one sample of a milk of bad quality, as many as 124 million bacteria were present in every cubic centimetre. In ordinary water, however, the amount of food material is not sufficient to support such large numbers. In ascertaining whether water has been polluted or not, the usual method of examination is to find out how many bacteria are present in the water. The following table indicates the standard accepted by many bacteriologists :

	Bacteria per c.c.
Very pure water,	0–50
Good water,	50–500
Passable water,	500–3,000
Bad water,	3,000–10,000
Very bad water,	10,000–100,000 and over.

If a large number of bacteria is present, the inference is that organic material must be present in sufficient quantity to support them, hence, drinking water containing large numbers of bacteria must be regarded with suspicion. If further examination shows that the organisms are such as are usually found in sewage water, the water is condemned for drinking purposes. It is necessary to make a qualitative as well as a quantitative examination, because the danger arises not so much from the presence of the bacteria themselves, as from the poisonous excretions of the harmful ones. Thus we find in ordinary pure water such organisms as Bac. subtilis, Bac. prodigiosus, Bac. rubescens, Bac. mycoides, Bac. aquatilis, also some species belonging to the Coccaceae and Spirillaceae, which are quite harmless, and no count is taken of them unless they are present in very large numbers. On the other hand, if members of the Proteus family, or Bac. enteritidis sporogenes, or Bac. coli communis are found, sewage contamination is indicated, and further, it may reasonably be suspected that, in addition to these, other organisms are possibly present which excrete poisonous substances, the most dreaded being the bacillus of typhoid fever and that of cholera. With regard to the different seasons of the year, the following table is interesting as showing the difference in the number of bacteria in rivers at different times of the year.

River Thames: water collected at Hampton:

	1 c.c. Water contained
January,	92,000
February,	40,000
March,	66,000
April,	13,000
May,	1,900
June,	3,500
July,	1,070
August,	3,000
September,	1,740
October,	1,130
November,	11,700
December,	10,600

These figures must not be regarded as giving absolute values for all rivers, for they do not always hold for the same river at different places, but the relative values can be accepted for most of the rivers in this country. The figures show that river water contains far more bacteria in winter than during the rest of the year. This is due to the fact that during the winter months, the rivers receive the washings

of the soil in larger quantities than during the remainder of the year, and, as will be seen presently, small quantities of soil usually contain many thousands of bacteria. When the water of a river flowing through a town becomes contaminated, as generally happens, especially if the sewage of the town be allowed to flow into the river, the bacterial content of the water becomes enormously greater. The following table shows how the Severn is affected by its passage through Shrewsbury:

	Bacteria per c.c.
Two miles above Shrewsbury, - - -	7,000
Waterworks opposite Shrewsbury, - -	13,000
Ferry I., 0·6 miles lower down, - - -	20,000
English Bridge, 1·6 miles lower down, - -	23,000
Ferry III., 2·5 miles lower down, - - -	19,000
Uffington, 4·7 miles lower down, - - -	17,000
Alcharn, 9 miles lower down, - - -	13,000
Cressage, 16 miles lower down, - - -	5,000

This table also shows that rivers can purify themselves, for we see that, a few miles below Shrewsbury, the Severn has returned to its normal conditions. It is important to understand the means at the disposal of rivers whereby purification is effected. The four chief agencies are oxidation, dilution, deposition, and competition among the different organisms. The first cuts off the food supply, and bacteria cannot multiply unless the necessary organic pabulum is present. When this becomes oxidised, it is rendered unfit for consumption by bacteria, which consequently die of starvation. In the case of rivers, the water is oxygenated by absorption at the surface, this process being facilitated by the movement of the water, whilst weirs and waterfalls cause a considerable increase in the amount of oxygen in the water. The effect of the second agency is seen when it is considered that the sewage and other liquid pollutions come into contact with a greater volume of liquid as they move down with the river, so that the number of organisms per unit volume becomes less. The third agency, viz. deposition, is a very important factor, for, as the sewage is carried down, all the particulate matter, sooner or later, sinks to the bottom. It might appear that the bed of the river would thus become, in course of time, an obnoxious breeding place for bacteria, but such is not the case. The bacteria have to enter into competition with other organisms which require the same organic pabulum. In this way water plants, especially the lowest forms, the algae, and the minute animals that are found in the water, make great inroads on the organic pabulum, with the result that the food supply of the bacteria is cut off. In addition,

mention must be made of the effect of light, which has been proved to have a germicidal effect on bacteria. In the case of rivers, however, its contribution to the diminishing of the number of bacteria is small in comparison with the other agencies.

The number of bacteria in pools and other stationary waters is dependent on the nature of the bed, the depth of the water, and the presence or absence of germicidal substances in the water. A surface water may contain many thousands of bacteria per cubic centimetre, whereas deep well waters and spring waters contain very few bacteria, for these waters have very little opportunity of becoming contaminated. In reservoirs and similar waters, where there is no motion, there are very few bacteria, for, however impure the water may have been when carried into the reservoir, sedimentation soon removes the particulate matter, and with it almost the whole of the bacteria. Thus, the water supplied to London, which is stored in reservoirs before being carried to its destination, contains, when it is consumed, less than 20 bacteria per cubic centimetre. The water supplied to Glasgow (from Loch Katrine), which also is allowed to have the benefit of sedimentation in large subsidence reservoirs before consumption, contains even less than this small number. The greater facility for sedimentation results normally in the presence of a very small number of bacteria in lakes, and, speaking generally, the deeper the lake the smaller will be the number of bacteria per cubic centimetre. Bacteria are also to be found in the sea, the number present in any particular locality being dependent on the amount of organic matter in the water. Consequently, we find that the greatest number is to be found near the land, and that the number diminishes rapidly further out to sea. As the sea bottom contains a large amount of organic remains, bacteria are there present in considerable numbers, and currents sometimes bring them up to the surface from a depth of as much as 600 fathoms. They have been found in large numbers at a depth of 100-200 fathoms. As a class such bacteria are generally motile, and the majority belong to the Spirillaceae.

To estimate the number of bacteria in any particular sample of water, a small quantity, diluted if necessary, is added to liquefied nutrient-gelatine or nutrient-agar contained in a Petri-dish. After cooling, the Petri-dish is set aside until the colonies appear. These are then counted: as each of the microbes placed on the Petri-dish grows into a colony, the number of colonies gives the number of bacteria that was present in the small quantity of the sample that was transferred to the Petri-dish.

§ 3. BACTERIA IN THE SOIL.

The number of bacteria in the soil is usually enormous, because not only is organic material normally present, but also most of the soil-bacteria belong to the spore-forming kinds, so that when food-stuff is not available, or sufficient moisture be not present, they are able to assume a resting state in the spore-condition until such time as more favourable conditions hold. The bacterial contents of an ordinary soil may range from 10,000 to 5,000,000 per gram, whilst polluted soil is, like milk and sewage-water, an ideal nutrient medium, and consequently can support an enormous number of bacteria. In such soils as many as 100,000,000 and more per gram may be found. It must be borne in mind that the soil is the depository of the remains of animal and vegetable organisms, and further that these are constantly undergoing changes at the hands of a beneficent nature, to the end that they may once more become useful to future generations of plants and animals. Nature's agents in this are almost all of a bacterial kind. Virgin soils have fewer bacteria than those that are cultivated, whilst the latter have fewer than prepared soils. This must inevitably result, because in prepared soils the fertilisers that are used serve as excellent food-material to different kinds of bacteria, and, in fact, this is the object of the cultivator, for the higher plants cannot utilise the fertiliser until the composition of the latter has been changed by the activity of bacteria. The number of bacteria in the soil of densely populated places—whether populated by man or the lower animals—is much greater than in that of sparsely populated localities, owing to the larger amount of organic material resulting from the greater number of deaths, the greater amount of waste-food, excretory matters, etc. The number of bacteria rapidly diminishes as we descend from the surface. Below 5 or 6 feet only a small number of anaerobic bacteria are generally found, and 10 feet down the soil is practically sterile, for at this depth there is no organic material to support bacterial life.

With regard to the different kinds of bacteria that are found in the soil, there are saprophytic bacteria, which live on dead organic matters, nitrite- and nitrate-bacteria, which feed on ammonia-compounds and nitrites respectively, also nitrogen-bacteria, which win back from the atmosphere the nitrogen gas which, in the free condition, has been given off during the decomposition of various organic matters. Again, denitrification bacteria are present. These break down nitrates, and

are the chief cause of the liberation of free nitrogen into the atmosphere. Finally, we unfortunately find pathogenic or disease bacteria, the chief being the bacilli which cause the diseases known as lock-jaw, quarter-evil or black leg, and malignant oedema. These are natural to the soil and form spores, so that they are able to retain their vitality unimpaired for a long time when the conditions are not favourable for their growth. At the same time it must not be imagined that the bacteria which cause other diseases are absent from the soil. Thus, a number of typhoid bacilli find their way to the soil owing to the deposition of sewage and animal excreta on its surface. It has been shown that in certain soils this microbe can remain alive for four hundred and four days after being placed on the surface. Again, the bacilli of tuberculosis and other diseases find their way to the soil occasionally, and, though not isolated, the microbe causing diarrhoea is probably normally present in the soil. There are some soils which are free from bacteria. These are the waste places of the earth, where the conditions are such that bacterial life is not possible owing to want of moisture or of organic pabulum. Such places are, for example, the great sandy wastes of the world which do not support life in any form, in fact, where other kinds of life are impossible those places will also be devoid of bacterial life.

As to the method of examining the bacterial contents of a soil, a very simple method consists in mixing a known quantity (about $\frac{1}{4}$ gram) of soil with a known quantity (about 50 c.c.) of sterile water, and after thoroughly mixing the soil and the water, to pour about a quarter of a cubic centimetre of the mixture into a Petri-dish containing liquefied nutrient agar or gelatine. The bacteria contained in the mixture of soil and water are allowed to grow into colonies, which are counted. Thus, suppose we found 200 colonies on a plate :

$\frac{1}{4}$ c.c. of mixture of soil and water contains 200 colonies,

$\therefore$ 50 c.c. of mixture of soil and water contains $200 \times 4 \times 50$ colonies.

Now, 50 c.c. contain $\frac{1}{4}$ gram of soil,

$\therefore$ $\frac{1}{4}$ gram of soil contains $200 \times 4 \times 50$ bacteria,

$\therefore$ 1 gram of soil contains $200 \times 4 \times 50 \times 4 = 160{,}000$ bacteria.

This number will not include the anaerobic bacteria. To ascertain their number the same method may be used, but cultivation must be effected under conditions in which oxygen is excluded.

CHAPTER VII.

STERILISATION.

An object is said to be sterilised when it has been freed from living germs. It must be remembered that, as a rule, the air is charged with spores of bacteria and fungi, and with the dried but living bacteria themselves, and these being subject to gravity fall to the ground; therefore every article is covered with them except in those places where bacteria are non-existent, as at the summit of Mont Blanc, or in the Arctic and Antarctic regions.

As these organisms will germinate rapidly, if provided with the necessary moisture and food material, it is necessary to free from them any object which we intend bringing near or into contact with a nutrient medium; for they will drop in and soon make the medium useless for the cultivation of other bacteria. In cases of infectious diseases it is manifestly important to remove the bacteria causing these diseases away from our persons, so that our clothes, all materials that come near our bodies, the surrounding atmosphere and walls must be treated in such a way that the risk of infection is diminished. In freeing an object from bacteria, care must be taken that it is not destroyed altogether for the purpose we have in view. Thus, objects like gelatine and cotton wool cannot be sterilised by dry heat, for they would soon get charred and become quite useless; hence, it is important to know the best method of sterilising any particular substance that we wish to free from germs.

Sterilisation of Air. To procure sterilisation, it is essential that the disinfectant be brought into immediate contact with the bacteria. It is a very common error to imagine that, if a dish of carbolic acid be placed in a room, the room is thereby freed from germs. There is no doubt that all the bacteria which actually fall into the dish will be rendered harmless; but this number is extremely small compared with

the number that do not fall into the dish, and which are scattered over the walls and various objects in the room. It cannot be expected that these dishes of disinfectant have the power of luring the bacteria from every part of the room into the carbolic acid, as serpents are said to lure birds to their destruction, by the power of fascination.

If we remember that actual contact is necessary, the absurdity of this idea is evident. The same applies to solid disinfectants meant to be hung on the walls of a room. From these, it is supposed, there emanates an odour deadly in its effect to all the germs in the neighbourhood. It may at once be stated that no gas is a disinfectant, and still less is an odour, however much it smacks of coal tar. Obviously, therefore, if we wish to purify a room, our only chance consists in spraying the walls, the floor, and the objects in the room, with a reliable liquid disinfectant. This tends to purify the places from whence the air derives its supply of bacteria. In course of time, the bacteria that were in the air when the spraying took place, will fall under the influence of gravity, and be subjected to the influence of the disinfectant. This will tend to purify the room, but, as seen in what follows, bacteria have a high degree of resistance, especially in the spore condition, and the spraying must be thorough, and, if possible, renewed several times after definite intervals. As to the choice of a liquid, regard must be paid to the following conditions:

1. Efficiency as a disinfectant.
2. If efficient, the lowest percentage of solution in which it is effective.
3. Cost.
4. Effect on the substances to be sterilised.

We shall consider these again, after we have dealt with the various disinfectants.

Another method of sterilising the air in a large chamber is to filter it as it enters. This method depends on the fact that there are various substances which allow air to pass through, but which retain the enclosed germs. Cotton wool is a filter of this description. A cotton wool filter is employed to purify the air admitted to the sterilised wort in an apparatus for the pure cultivation of yeast. Such filters, however, can be considered reliable only provided the filter be dry, for if wet, the entangled bacteria that the cotton wool has stopped will grow through the filter, and thus contaminate the atmosphere which it is desired to purify.

Another method of sterilising the atmosphere of a room, is to allow the air, as it comes in, to impinge against a wet surface before entering

the room. The bacteria are caught and are in much the same position as flies in a bowl of milk.

Disinfectants. We must distinguish between an *antiseptic* and a *germicide*, the latter being used to designate an agent that kills bacteria, whereas the former applies to one which hinders their development. It is like the difference between merely stunning a man and killing him outright, and naturally, the latter is the more effective of the two. But, just as a stunned man may recover his senses, so, it must be borne in mind, may the bacteria, treated with antiseptics, recover their strength and multiply, should they find themselves in a suitable medium.

It will be well, first of all, to consider the conditions that are inimical to bacterial life. These are:

1. Very high and very low temperatures.
2. Light.
3. Chemical substances that act as poisons.
4. Very long continued dearth of water.

Two of these, however, cold and drought, may be dismissed at once as practical disinfectants, on account of the very long time which would be required for dearth of water or want of heat to result in destruction of bacteria. As, however, no multiplication of bacteria could take place under these conditions, they may be regarded as slight antiseptics. Thus, it has been shown that the tubercle bacillus can stand the temperature of liquid air (-193° C.) for 42 days without losing its vitality; and the spores of bacteria have been found to possess the power of germination after being in a dry condition for several years.

Heat, however, is a practical disinfectant. When the temperature of protoplasm reaches 55° C., in the case of all except the *thermophilous* or heat-loving bacteria, death ensues. Also, in the case of even the thermophilous kinds, the death-temperature is not very much higher; hence, we have only to heat the substance until conduction has effected a sufficient rise of temperature in the protoplasm. As the temperature of boiling water at normal atmospheric pressure is 100° C., sterilising by its means is very effective in the case of non-sporogenous bacteria, to which, fortunately, almost all the pathogenic bacteria belong. It is far more difficult, however, to kill bacteria when they are in the sporogenous condition, for the outer of the two coats of the spore is an extremely bad conductor of heat, so that much more prolonged heat is required for efficient sterilisation.

The following table shows the difference between the resisting power of bacteria when in the sporogenous and when in the non-sporogenous

condition. Material containing bacteria was placed in boiling water. The figures indicate the time required to destroy the bacteria:

	Bacteria in Spore Condition.		Bacteria in Vegetative Condition.
Bac. tumescens, - -	4–5	minutes.	All killed in
,, cohaerens, - -	4·5–5	,,	2 minutes.
,, simplex, - -	3–4	,,	
,, myeoides, - -	10	,,	
,, pumilis, - -	6–7	,,	
,, fusiformis, - -	3–4	,,	
,, carotarum, - -	4·5–5·5	,,	
,, Ellenbachensis, -	1–2	,,	
,, graveolens, - -	7–10	,,	
,, subtilis, - -	150–180	,,	
,, ruminatus, - -	1·75–2	,,	

Although in the case of Bac. subtilis continuous heating for 150-180 minutes at 100° C. was necessary in order to incapacitate it for germination, it must not be thought that the protoplasm of this organism is more resistant than that of other bacteria; the greater resistance is due to the greater efficiency of the spore-coats. The method of *intermittent sterilisation* is based on a knowledge of the difference in resisting power of bacteria in the spore- and in the vegetative-condition. A nutrient medium is placed in a steam-steriliser for about 20 minutes on three successive days. On the first application of heat all the vegetative cells are killed, but probably not all the spores. During the next 24 hours the surviving spores, being in a good nutrient medium, germinate: when the nutrient medium is now again placed in the steam-steriliser, the germinated cells are quickly killed. On the third day, after the third application, the medium may be regarded as sterile.

We must now describe the steam-steriliser. This is shown in Fig. 65, which represents the first kind in use, known as Koch's Steam-steriliser. It consists essentially of a chamber with a receptacle at the bottom for holding water (*A*). This latter is separated from the chamber by a detachable, perforated, tin disc (*B*). The tap at the side is for pouring out the water when required, and the arrangement at *C* enables the operator to find out how much water is contained in the receptacle. The object to be sterilised is placed on *B*, and heat is applied by placing a burner underneath. The chamber is soon filled with steam, and the object attains the same temperature as the steam. The great defect of this kind of steriliser is that the steam escapes into the

surrounding atmosphere, and thus the room is apt to become too damp, a condition which is not good for a bacteriological laboratory. In order to prevent the dissipation of heat by radiation, Koch's steriliser is

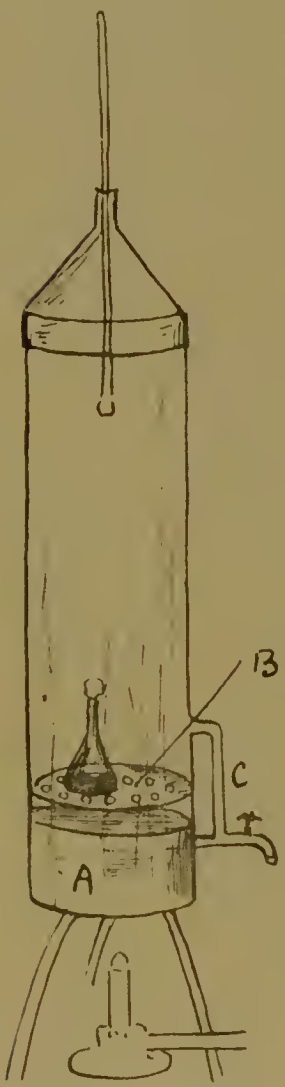


Fig. 65.—Koch's Steam-steriliser.

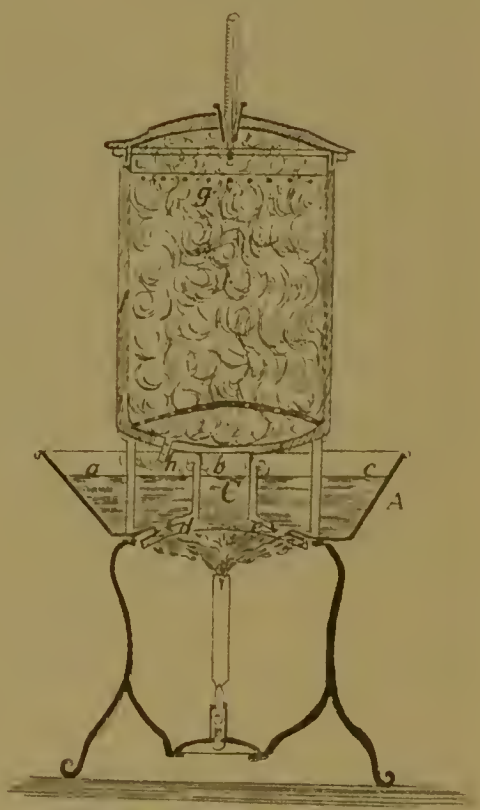


Fig. 66.—Lümkemann's Steam-steriliser.

usually covered with a coat of thick felt round the sides, and a cap of the same material is placed on the cover.

A far better steriliser is that of Lümkemann, because it condenses almost the whole of its own steam, thus preventing the large escape of the latter into the room. Fig. 66 illustrates this apparatus, which is usually made of copper. The burner is applied underneath the shallow pan *A*, which contains water. It will be noticed from the

diagram that the water is in communication at *a*, *b*, *c*, *d*, and *e*, hence the water at *a*, *b*, and *c* will always be at the same level, and the supply of water for the steam is drawn from the water in the pan *A*. The steam issuing from *C* is carried up between the two walls of the apparatus (*f*) and at the top enters the chamber through small apertures in the inner wall (*g*). The chamber thus becomes filled with steam. As shown in the diagram, the bottom of the chamber also has two walls, and the inner of the two communicates with the pan *A* by means of the tube (*h*), so that all the steam condensed in the chamber drops back into the pan *A* in the form of water, instead of escaping into the room in the form of vapour.

In both forms of sterilisers the objects to be sterilised are usually placed in wire cages, either round or rectangular so that they can be easily lifted in and out. By this method of moist heat all nutrient media, all liquids, and all substances which cannot stand a higher temperature than 100° C. without being spoilt, are sterilised. This is the most convenient method of freeing dusters, articles of clothing, etc., from germs.

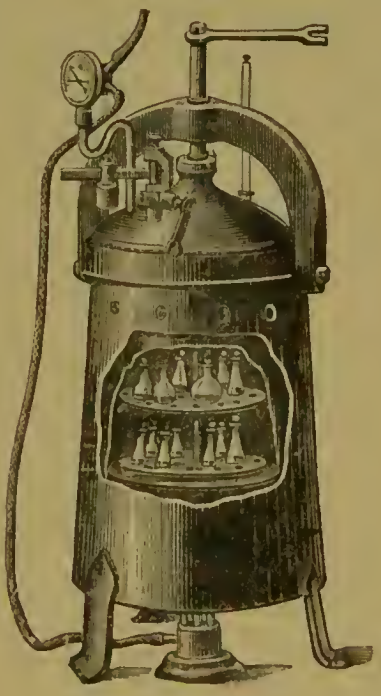


Fig. 67.—Autoclave.

Sterilisation by moist is more efficacious than by dry heat, but a higher temperature is possible by the latter method, and is, therefore, preferable for substances that can stand much heat without injury. Again, in the case of cotton wool, sterilisation must be effected by dry heat, for moist cotton wool used as a stopper for a flask containing a nutrient medium is ineffective. Hence, all glass ware and cotton wool are sterilised by being placed in the dry-air oven, which must be heated for at least 20-30 minutes at 120° C.

We may use the naked flame of a Bunsen burner to sterilise knives, platinum loops, platinum wires, glass rods, and other substances not injured by coming into contact with the naked flame.

Finally, a very efficient heat method is by the use of the *Autoclave*. The principle of this apparatus depends on the fact that the boiling point of water depends on the pressure. In the ordinary steam-steriliser the water is heated at the atmospheric pressure at which

the temperature of boiling water is 100° C. If, now, the pressure is in some way raised water will not boil until a higher temperature is attained. In the autoclave increased pressure is obtained by preventing the escape of steam. Otherwise it is the same in principle as the Koch's steriliser. The apparatus must naturally be made very strong, and the lid is made of some thick metallic substance with a special appliance for keeping it down when the pressure rises. The apparatus is shown in Fig. 67. For the practical working of this apparatus the reader is referred to Arthur Meyer's *Practicum der botanischen Bakterienkunde*. The sterilisation is most effective, as even the most resistant of spores, *e.g.* those of Bac. subtilis, are killed after about 20 minutes of 120° C. of moist heat. The autoclave dispenses with the necessity of heating the object on three successive days.

The effect of the autoclave on the most resistant of spores is shown in the following table:

At 105°–110° C., - - -	killed in 2–4 hours.
,, 115° C., - - - -	,, 30–60 minutes.
,, 120° C., - - - -	,, 5–15 ,,
,, 125°–130° C., - - -	,, 5 ,,
,, 140° C., - - - -	,, 1 ,,

In one experiment it was found that the extremely resistant spores of the potato bacillus succumbed in the following times:

At 100° C., - - - -	after 5½–6 hours.
,, 109°–113° C., - - -	undecided, some still alive after ¾ hour.
,, 113°–116° C., - - -	after 25 minutes.
,, 122°–123° C., - - -	,, 10 ,,
,, 126° C., - - - -	,, 3 ,,
,, 127° C., - - - -	,, 2 ,,
,, 130° C., - - - -	instantaneously.

These results suffice to show the efficiency of this apparatus.

If we turn now to *light* as a means of sterilisation we find that whilst it has been proved that continual exposure to light is fatal to bacteria, yet as a practical disinfectant it cannot be used owing to the long time required before the bacteria can be killed. In the case of spores it is doubtful whether their capacity for germination is at all affected. The spores of soil-bacteria, for instance, must be exposed for very long periods to the influence of sunlight.

Finally, we have to consider various chemical substances that act as disinfectants. We must consider the qualities that make a good disinfectant. Andrews has laid down the following:

1. It should be germicidal within a reasonable time limit.
2. It should not possess chemical properties which make the disinfected substances unfit for ordinary use.
3. It should be soluble in water, or capable of giving rise to soluble products in contact with the material to be disinfected.
4. It should not produce injurious effects on the human tissues.
5. It should not be too costly in proportion to its germicidal value.

The following chemicals have been recommended:

Mineral Acids. These include sulphuric-, nitric-, hydrochloric-, and phosphoric-acids. There are very few bacteria that can live even in a slightly acid medium. Thus, in the case of a weak acid like phosphoric acid, one drop of a 1·75 per cent. solution added to 5 c.c. of broth inoculated with the spores of Sarcina ureae, is sufficient to make the medium quite unsuitable for the development of this germ. The application of mineral acids is limited because of their disastrous effect on the skin, and their corroding action on metallic substances. Also, as is well known, acids destroy all cloth substances. They are useful when we wish to sterilise small quantities of liquid containing pathogenic bacteria before throwing the liquid away.

Alkalies, *e.g.* potash (KOH), sodium hydrate (NaOH), etc. Most bacteria can thrive in very slightly alkaline media, but growth is prevented when the reaction becomes decidedly alkaline. The effect of a 5 per cent. soda solution on the spores of Sarcina ureae is shown by the following experiment: Three drops of this solution were added to 5 c.c. of broth inoculated with this organism. This was sufficient to prevent the normal formation of spores, though at first growth without spore-formation was not prevented. After five days the conditions were so unfavourable that all the individuals in the culture had succumbed. We may then say that all strong alkalies are disinfectants, but that as they prejudicially affect the skin and clothes, like acids, they can only be advantageously used to sterilise infected substances previous to throwing them away.

Carbolic Acid. Probably this is the best known disinfectant on account of its effectiveness, its cheapness, and absence, in the diluted condition, of injurious action on the clothes and skin. A strength of 1 in 40 has been found sufficient to destroy Streptococcus pyogenes, S. erysipelatis, and Staphylococcus pyogenes aureus, whilst tubercular sputum has been sterilised by being mixed with a 1 in 20 solution,

after one minute. As a wash for the hands it is sufficient to use a 3 per cent. solution, and a 5 per cent. solution will effectively sterilise objects immersed in it.

Formalin. This substance is a 40 per cent. solution of formaldehyde. It can destroy the non-sporogenous cells of typhosus, anthrax, and cholera in a very weak solution. It is claimed that a teaspoonful of it is sufficient to prevent the souring of 10 gallons of milk. The substance called *paraform* is the white residue obtained by the evaporation of formalin. This powder is made up into lozenges, which are used in the preparation of formalin fumes by being burnt in methylated spirits; the fumes are easier to obtain from paraform than from formalin itself. Of late years several contrivances have appeared on the market to facilitate the production of this vapour.

1. *Sprayer.* This produces a mixture of air and fine particles of the solution, and is useful for spraying walls, floors, and sometimes garments. There are several forms on the market.
2. *The Autoclave* (Trillat's apparatus). In this apparatus a mixture of 30-40 per cent. aqueous solution of formalin and 4-5 per cent. calcium chloride is heated under pressure of 3-4 atmospheres. The vapour is usually passed through the keyhole of a sealed-up room, which is thus subjected to a vapour in all its parts.
3. *The Paraform Lamp* (the Alformant). Paraform in the form of tablets is subjected to the action of the vapour produced by the combustion of methylated spirit. When saturated with paraform, this vapour is a very effective disinfectant.
4. *Lingner's Apparatus.* Steam is generated in a ring boiler and then driven into a reservoir containing formalin or glycoformal (30 per cent. formalin and 10 per cent. glycerine). The resulting vapour is ejected in the form of a fine spray through four nozzles, and fills the room with a thick vapour. Houston and Newman found that after 4 hours' exposure Bac. pyocyaneus, Staphylococcus pyogenes aureus, and other organisms were destroyed. Klein and Newman also found that Bac. anthracis and the bacillus of consumption were killed by the same means. It has also been claimed that anthrax spores can be killed by vapourising per cubic metre 7·5 c.c. of formalin with four times its volume of water, the exposure which is necessary being not more than 6 hours.

It is obvious, then, that we have in formalin an extremely useful disinfectant. The objection to it in the vapour condition is that it has an irritating effect on the mucous membrane of the nose and

throat, and causes the eyes to smart. But its penetrating power is greater than that of most disinfectants. Delepine proved that formalin was fatal to Bac. tuberculosis, Bac. pyocyaneus, and Staphyloeoccus pyogenes aureus, even when these organisms were protected by three layers of filter paper.

Mereuric Chloride (corrosive sublimate). This antiseptic is so efficient that 1 in 14,300 is sufficient to sterilise most substances. In the fermentation industries its use is out of the question owing to its intensely poisonous properties, but in the laboratory it is invaluable for washing wounds, disinfecting bandages, etc. Glass ware and cultures of bacteria that have been put aside may also be treated with this powerful germicide; but care should be taken that glass utensils thus treated are thoroughly cleansed from the chloride; especially is this necessary with tubes that may again be required for bacterial cultures. The strength which is recommended is 1 gram per 1 litre of water. The water must not contain any calcareous matter, and it is also advisable to add 5 grams of common salt per 1 litre of disinfectant to prevent the corrosive sublimate from forming insoluble compounds with albuminoids, and so prevent its action as a disinfectant. Many experiments have been made to demonstrate the efficiency of this compound. Bac. typhosus and the cholera spirillum, growing in flesh-peptone-gelatine are destroyed in 2 hours by a solution of 1:10,000. The anthrax bacillus can be killed by a 1:100,000 solution at 36° C., but if the temperature be lowered to 3° C., a 1:25,000 solution is required. In experiments upon tuberculous sputum, when fresh, a solution of 1:2000 acting for 24 hours, failed to kill the contained bacteria, but a 1:1000 solution succeeded after 1 minute.

Sulphurous Acid. The practice of burning sulphur as a means of disinfection has been known probably as long as the art of making wine. The burning of sulphur produces sulphur dioxide, and when the latter is dissolved in water, sulphurous acid is produced. The cheapness of this method, and the ease with which it can be carried out, has made it very popular for purposes of disinfecting utensils, clothing, etc.; but, according to the most recent investigations, its disinfecting power is not very great. Sulphur fumes have little or no effect on most bacteria when in a dried state, but when in a moist condition, if they have not formed spores, they can be destroyed by such means. There is one exception, however, viz., the tubercle bacillus: this organism, though it does not form spores, is very resistant, and, if its presence is suspected, sulphur fumes should not be used. Three to six pounds of sulphur are generally used for every

1000 cubic feet of space. From what has just been stated, it is obvious that the walls, floors, etc., must be sprayed with water preparatory to being subjected to the action of the fumes. The room should be kept closed for at least 24 hours. The employment of sulphur fumes, under pressure, is probably the most convenient method for the disinfection of ships.

In the fermentation industries this method of disinfection is very extensively used. The following table shows how far sulphur dioxide in solution is effective in the destruction of yeasts and other fungi:

Species.	Fatal dose Sulphur Dioxide in c.c. per litre for an exposure lasting			
	15 mins.	6 hours.	24 hours.	5 days.
Beer Yeast, - -	200	100	20	...
Wine Yeast, - -	100	20	20	10
Mycoderma vini, -	200	100	100	40
Aspergillus niger,	50	20	10	...

According to Linossier, 25 c.c. of sulphur dioxide per litre is sufficient, not only to prevent fermentation from making a start, but also to stop it when it has started. The use of sulphur dioxide in the gaseous form is not often resorted to in the fermentation industries, because it attacks the metal fittings, and irritates the lungs of the workmen. In breweries the use of calcium bisulphite ($Ca(HSO_3)_2$) in disinfecting the fermenting tuns is very extensive.

Milk of Lime or Calcium Hydroxide is often employed to wash the walls of malt-houses, etc. It is fairly good when fresh, but, when it has stood for some time, it becomes converted into the carbonate which has no injurious effect on micro-organisms. The reaction which takes place is as follows:

$$\underset{\text{calcium hydrate}}{Ca(OH)_2} + CO_2 = \underset{\text{calcium carbonate}}{CaCO_3} + H_2O.$$

According to one observer, the typhoid bacillus and the cholera-spirillum in broth cultures are killed in four or five hours by the addition of 0·1 per cent. of calcium oxide. Again, the dejections of typhoid patients can be sterilised in six hours by the addition of 3 per cent. of this substance, and, when the percentage is raised to 6, in two hours. The practical value of the application of lime-wash to

walls has been determined. Silk threads, soaked in cultures containing various pathogenic organisms, were attached to boards, and lime-water applied with a camel's-hair brush; anthrax bacillus (without spores) and some others were killed, after a single application, in twenty-four hours, but three applications were not sufficient to kill the tubercle bacillus. The spores of anthrax were not killed by two hours' exposure to milk of lime containing twenty per cent. of calcium oxide. We may regard this disinfectant as being very useful, except in cases where it is suspected that very resistant organisms are present. It has the further advantage of being harmless to the human system, and can therefore be freely used in breweries, malt-houses, etc.

Boracic Acid. Bandages for wounds are usually sterilised by soaking them in boracic acid. It has also been employed for preserving tinned meats, milk, etc.; but as cases of cumulative poisoning have been traced to this substance, its use as a food-preservative is now forbidden by law.

With regard to exact determinations of its value, it has been found that a 2·7 per cent. solution can destroy the bacillus of typhoid in 5 hours, and a 1·5 per cent. solution can do the same to the cholera bacillus in the same time. Anthrax spores cannot be killed by boracic acid; in fact, it has been found that a 5 per cent. solution was unable to destroy them, even after 5 days' immersion. Its use, in the form of borax, is recommended in the preparation of starch paste, because it prevents the paste from being attacked by moulds.

Hydrogen Peroxide. The destructive effect of this substance is not in virtue of its own poisonous effect on the living matter of bacteria and allied organisms, but rather owing to the fact that it liberates oxygen easily, and it is the oxygen which destroys. The price of this article is too prohibitive to enable its use to become general, otherwise, in the fermentation industries, there would probably be a great demand for it, inasmuch as it can easily be made to split up into water and oxygen, both substances harmless to the human system. The equation is expressed thus:

$$\underset{\text{hydrogen peroxide}}{H_2O_2} = \underset{\text{water}}{H_2O} + \underset{\text{oxygen}}{O}.$$

In the manufacture of jams, pickles, etc., its use is strongly recommended. Its value to the medical profession is still a debated point. Miquel, in his list, dubs it a substance eminently antiseptic, and states that, even in the proportion of 1 : 20,000, the development of germs is considerably hindered. Sternberg's conclusion is that, unless some

means are discovered to furnish more concentrated solutions of this antiseptic, and other means to prevent its decomposition, which takes place very easily, its use as a practical disinfectant cannot be regarded as satisfactory. Later experiments have shown that Miquel's estimate of 1 : 20,000 is too high, as Altehofer's experiments proved that the ordinary water-bacteria and several of the pathogenic bacteria experimented upon, required for their destruction an exposure of twenty-four hours in a solution containing hydrogen peroxide, in the proportion 1 : 1000. This considerably lowers the value of hydrogen dioxide as a disinfectant.

Carbonic Acid Gas or Carbon Dioxide. Several experiments have been made to test the value of this gas in preventing the growth of bacteria. In many of them it is impossible to say whether the absence of growth was due to the absence of oxygen, or to the presence of carbon dioxide, but, according to Fränkel, a number of anaerobic bacteria were hindered in their growth by the presence of this gas. Again, two species of spirillum failed to develop under the same conditions, and after eight days, when air was again introduced, the two species were no longer capable of growth. In this last experiment the deprival of oxygen for eight days might have been the cause of death. For practical purposes, carbon dioxide is not strong enough to be regarded as an efficient disinfectant.

Potassium Permanganate. According to Miquel, 1 : 285 is the proportion in which this substance begins to be effective.

The following results indicate its efficiency :

1. Pus cocci were killed in two hours by immersion in a 1 : 833 solution.
2. Anthrax spores were killed after 24 hours' immersion in a five per cent. solution, but a one per cent. solution failed even after two days.
3. A five per cent. solution was ineffective for the tubercle bacillus.

The disinfecting quality of this chemical depends on its property of liberating a part of its oxygen very readily. When oxygen is in the nascent condition, that is, immediately after its liberation, it possesses powerful germicidal properties. Condy's fluid is largely made up of this disinfectant.

Sodium Chloride or Common Salt. Common salt, in the proportion 1 : 6, was formerly supposed to be an active germicide. Later, more accurate experiments have not borne out this supposition. For example, a saturated solution of common salt failed to destroy anthrax

spores, even after 40 days, and it was also ineffective when tried against the tubercle bacillus. Further, even the more susceptible Bac. typhosus could not be killed after several weeks' exposure in strong solutions of common salt. The still more susceptible spirillum of cholera, however, succumbed after a few hours' exposure. The value of this article lies in the fact that no development of micro-organisms can take place in substances covered by or immersed in it; it is a poor germicide, but an excellent antiseptic. From this it follows that the consumption of diseased meat, even though salted for some time, is fraught with the greatest danger.

In addition to the gases and vapours already dealt with, we may add the following remarks with regard to others:

Oxygen is a disinfectant only in the nascent condition.

Ozone is of no practical value. In one experiment Bacillus anthracis, Staphylococcus pyogenes aureus and others, were exposed for 24 hours on silk threads in an atmosphere containing 4·1 milligrammes of ozone to one litre of air; all were quite unaffected by this exposure when subsequently brought into contact with nutrient media. This experiment puts ozone out of count altogether as a practical disinfectant.

Hydrogen is useless as a disinfectant.

Carbonic Oxide. Disinfecting powers of carbonic oxide, very slight and of no practical use.

Nitrous Oxide is of no practical use.

Nitrogen Dioxide. Several organisms tested by Frankland, viz. Bac. pyocyaneus, Spirillum cholerae Asiaticae, and Spirillum Finkler-Prior, were quickly killed by this gas.

Sulphuretted Hydrogen. The experiments with this gas by different observers give contradictory results, so we may safely assume that its germicidal value must be so small as to be of no use for practical purposes.

Chlorine. This gas has a strong affinity for hydrogen, and, as water is composed of hydrogen and oxygen, its combination with hydrogen liberates oxygen, which, in the nascent condition, is, as mentioned above, a powerful germicide. Hence, whilst dry chlorine is useless, moist chlorine is efficient as a disinfectant. Anthrax spores can be killed after exposure for one hour to an atmosphere containing four per cent. chlorine, and the same can be accomplished in three hours by a one per cent. solution. When the atmosphere and the spores were kept dry, a percentage of 14·7 of chlorine was of no use as a disinfectant. A culture containing the anthrax bacilli in the non-

sporing condition was destroyed by exposure for 24 hours to a moist atmosphere containing only ·0005 per cent. of chlorine. A further example of its excellent germicidal qualities is given by De la Croix's investigation, as a result of which it was shown that no development of bacteria or moulds took place in an unboiled meat infusion when chlorine, in the proportion of 1 : 15,600, was present in the atmosphere. Miquel, however, puts the proportion much lower, viz. 1 : 4000.

Mention has already been made of the germicidal properties of various acids. We must now deal with the more important.

Sulphuric Acid. Boer's experiments decided the proportion necessary, both to restrain the development of bacteria and also to destroy them when development had taken place. The time of exposure for all these experiments was two hours.

	To restrain development.	To destroy vitality.
Anthrax bacillus -	1 : 2550	1 : 1300
Diphtheria bacillus -	1 : 2050	1 : 500
Glanders bacillus -	1 : 750	1 : 200
Typhoid bacillus -	1 : 1550	1 : 500
Cholera spirillum -	1 : 7000	1 : 1300

This acid is therefore a powerful germicide, but unfortunately its use is limited to those substances which are to be disinfected before being thrown away. It is not cheap, it inflicts nasty burns on the skin and chars all organic substances.

Nitric and Hydrochloric Acids are both excellent germicides in themselves, but they are not cheap, and dangerous as well as unpleasant to use.

The following results obtained with hydrochloric acid, show the proportions of acid to water, which are necessary to prevent development of bacteria, and to destroy them outright.

	To restrain development.	To destroy vitality.
Anthrax bacillus -	1 : 3400	1 : 1100
Diphtheria bacillus -	1 : 3400	1 : 700
Glanders bacillus -	1 : 700	1 : 200
Typhoid bacillus -	1 : 2100	1 : 300
Cholera spirillum -	1 : 5500	1 : 1350

Phosphoric Acid. A 0·3 per cent. solution of this acid will destroy the typhoid bacillus in four or five hours.

Lactic Acid. It is important to know exactly the germicidal properties of this acid because of its extensive use in various

fermentation industries. Compared with other acids its antiseptic and germicidal properties are not very pronounced; thus the bacillus of typhoid fever is killed in five hours by a solution containing 0·4 per cent. of lactic acid. Its extensive use in the industries is due to the fact that its presence to a moderate extent in a liquid prevents the growth of a number of obnoxious germs, especially the butyric-acid bacteria. Consequently, in many industries it is found expedient either to inoculate such a liquid with lactic-acid bacteria or to place the liquid in such conditions that lactic-acid bacteria will multiply in it. In either case lactic acid is produced. Of course, in such industries the "souring" must not be allowed to go too far, otherwise the liquid becomes unfit for organisms of any kind to thrive in. The use of lactic acid in the various industries will be dealt with in greater detail when we come to deal with the industries that depend on fermentative processes.

Salicylic Acid. A two per cent. solution of this acid will destroy pus cocci in about two hours. Koch has tried the effect of this acid on anthrax spores. He dissolved it in oil or in alcohol and found that a five per cent. solution failed to destroy these spores. A 1·6 per cent. solution acting for five hours is sufficient to destroy the typhoid bacillus, and a 1·3 per cent. acting for the same time will do the same to the cholera spirillum. According to Miquel, it is an antiseptic in the proportion of 1 : 1000. In some industries salicylic acid is extensively used to prevent the formation of moulds in such substances as jams, wines, etc.

Butyric Acid. As a practical disinfectant this substance is out of the question, on account of its exceedingly unpleasant odour of rancid butter, but it has a practical interest for us, because of its possible production, due to the activity of butyric-acid bacteria, in brewing, distilling, butter-making, etc. If it be produced to any extent in a fermenting liquid, the latter is rendered useless. Fortunately, however, the toxic effect of butyric acid upon the activity of most bacteria is not great, and though it is without doubt an antiseptic, it is not more so than acetic, lactic, and other comparatively weak acids. Anthrax spores are unaffected even when immersed for five days in pure butyric acid.

The following table drawn up by Miquel, gives the minimum proportion for each particular substance in which growth of microbes is arrested. He obtained samples of all kinds of germs by collecting them where they were most abundant, viz. from dust of houses, and hospitals, from sewage-water, etc. Broth was inoculated with

germs from these samples. Then he ascertained the minimum amount of antiseptic necessary to prevent the broth from becoming putrid. We abstract from Miquel's tables the antiseptics that are of general interest, and it will be noticed that the various substances are arranged in order of their antiseptic strength.

Intensely Powerful Antiseptics.

	Efficient in proportion of		Efficient in proportion of
Mercuric iodide	1 : 40,000	Mercuric chloride	1 : 14,300
Silver iodide	1 : 33,000	Silver nitrate	1 : 12,500
Hydrogen peroxide	1 : 20,000		

Very Strong Antiseptics.

Osmic acid	1 : 6666	Hydrocyanic acid	1 : 2500
Chromic acid	1 : 5000	Bromine	1 : 1666
Chlorine	1 : 4000	Cupric chloride	1 : 1428
Iodine	1 : 4000	Thymol	1 : 1340
Chloride of gold	1 : 4000	Cupric sulphate	1 : 1111
Bichloride of platinum	1 : 3333	Salicylic acid	1 : 1000

Strong Antiseptics.

Potassium bichromate	1 : 909	Nitrate of cobalt	1 : 500
Potassium cyanide	1 : 909	Carbolic acid	1 : 333
Ammonia	1 : 714	Potassium permanganate	1 : 285
Zinc chloride	1 : 526	Lead nitrate	1 : 277
Mineral acids	1 : 500—1 : 333	Alum	1 : 222
Lead chloride	1 : 500	Tannin	1 : 207

Fair Antiseptics.

Arsenious acid	1 : 166	Salicylate of soda	1 : 100
Boracic acid	1 : 143	Ferrous sulphate	1 : 90
Arsenite of soda	1 : 111	Caustic acid	1 : 56
Hydrate of chloral	1 : 107		

Feeble Antiseptics.

Calcium chloride	1 : 25	Alcohol	1 : 10
Sodium borate	1 : 14		

Very Feeble Antiseptics.

Ammonium chloride	1 : 9	Glycerine (sp. gr. 1·25)	1 : 4
Potassium iodide	1 : 7	Ammonium sulphate	1 : 4
Sodium chloride	1 : 6		

These figures cannot be taken as absolute. The resistance capacity of bacteria is very variable. A species under some conditions may be three or four times more resistant than it is under other conditions. The same naturally applies to a collection of species, such as Miquel worked with. We must therefore allow for this and, for instance, interpret 1 : 4 as ranging between 1 : 6 and 1 : 2. The table, however, is valuable as indicating the average antiseptic value of various substances.

Finally, mention must be made of some of the interesting and homely substances which enter into the common round of daily life.

Alcohol. The germicidal value of alcohol has been greatly overrated. Thus Sternberg finds that even a 95 per cent. solution acting for 48 hours failed to kill bacterial spores. Absolute alcohol had absolutely no effect on anthrax spores, even when the latter had been immersed for 110 days. In the proportion of five parts of absolute alcohol to one part of tuberculous sputum, the latter was rendered innocuous only after 24 hours.

Camphor. This substance also has very little germicidal value as shown by the fact that such delicate organisms as typhoid bacillus and cholera spirillum were destroyed only after 8-10 days' exposure to the action of essence of camphor. The same remark applies to oil of camphor and tincture of camphor.

Coffee Infusion is an undoubted germicidal agent as evidenced by the following results:

1. A three per cent. solution restrained the growth of typhoid bacillus, and a five per cent. solution killed it after two days.
2. Cholera failed to grow in a one per cent. solution.
3. Proteus vulgaris (a common sewage organism) failed to grow in a nutrient solution containing 2·5 per cent. of coffee infusion, and was killed outright after two days, when the nutrient solution contained ten per cent. of this infusion.

These results are interesting and gratifying in view of the popularity of this palatable beverage.

Oil of Lavender, Eucalyptus, Rosemary, Cloves. These four substances are stated to be the best antiseptics among the essential oils.

Glycerine is not a good antiseptic, and in fact in small quantities it favours the growth of the bacillus of consumption. In the proportion of about 25 per cent. it hinders, but does not stop putrefactive decomposition.

Smoke. It has been demonstrated that it is only after six months' smoking, that meat can be said to be free from living germs.

Tobacco Smoke. It is interesting to note that it has been found that certain of the more susceptible bacteria, like the cholera spirillum, failed to develop in a nutrient medium after half an hour's exposure in an atmosphere of tobacco smoke. The good that tobacco smoke effects, does not however, by a ten-thousandth part, counteract the evil that is caused by the filthy habit of spitting that is so prevalent among the smokers of this country.

CHAPTER VIII.

ANAEROBIC BACTERIA.

When Pasteur in 1861 made known to the world that he had discovered an organism that could live and multiply without oxygen, the announcement was greeted with astonishment by the scientific world, and was followed by not a few expressions of incredulity. His assertions, however, were completely substantiated, and this opened the way to a new and interesting field of research. Pasteur's organism was described by him as one of the bacteria that were responsible for the butyric-acid fermentation of lactic acid, and named *Vibrion butyrique*. As the discovery was made before the days of pure cultures the organism was lost, but it is the same as that which was later described by Prazmowski under the name of *Clostridium butyricum*. It was found that the presence of oxygen was not only unnecessary, but actually harmful to this organism, for this gas put an end to the motility of the individuals and effectually prevented their growth and multiplication when introduced into a culture of these bacteria. The following simple experiment, first made by Pasteur on Vibrion butyrique, shows the behaviour of such organisms towards oxygen. A drop of the fermenting liquid was placed on a glass slide and examined under the microscope. The bacilli maintained their motility only at the centre of the drop, whereas nearer the edge the motility became less pronounced, and very near and at the edge soon ceased altogether. In consequence of his discovery Pasteur divided micro-organisms into two classes—the **aerobic**, which require, and the **anaerobic**, which do not require oxygen for their growth and multiplication. His publication naturally led to further researches on this subject, and it was soon discovered that the anaerobic bacteria were widely distributed and had important parts to play in the economy of nature. They are found in the deeper layers of the soil, in mud, in

excrement, and, in fact, in all those places where organic decomposition in the absence of oxygen is taking place. Most of them thrive on dead organic matter, and are therefore saprophytes, whilst a few are parasites, inasmuch as they thrive on living organisms. Of the latter class are *Bacillus oedematis maligni*, the cause of surgical gangrene, and *Bacillus tetani*, which is responsible for the lock-jaw disease. Among the saprophytes a few are important from an economic and industrial standpoint, though these have not as yet been carefully investigated.

Anaerobes do not all behave alike in the presence of oxygen. To some that gas is either fatal or a positive hindrance to growth; others, while flourishing best in its absence, are able to live and grow in its presence. The former are known as **obligate anaerobes** and the latter as **facultative anaerobes.** As will be presently shown, these remarks apply only when oxygen is present at the normal atmospheric pressure.

The facultative anaerobes do not show the same physiological characteristics when growing anaerobically as when oxygen is present. This is shown by the results of several investigators. Thus it has been demonstrated by one observer that Bact. formicicum under anaerobic conditions uses up salts of formic acid when these are presented to them in the nutrient medium, but under aerobic conditions these salts remain unchanged. Again, of the seven species of facultative anaerobes examined by another observer all, when growing aerobically, liquefied gelatine, but under anaerobic conditions all were devoid of this faculty. This points to the conclusion that when oxygen is presented to facultative anaerobes they are able to make use of it, and this conclusion has actually been verified experimentally; for by cultivating one of these organisms in an atmosphere containing a very small quantity of oxygen, it was found that the whole of the oxygen was used up.

Recent investigations, especially those of Beijerinck and Chudjakow, have profoundly modified our conceptions of the anaerobic bacteria. The results of the following experiment, taken from Beijerinck's researches, will show the trend of these changes. He poured a little sterilised nutrient-gelatine into a test-tube, added sterilised water to the gelatine, and then made an inoculation with an anaerobic bacillus. The water became turbid in consequence of the growth of this organism, but the turbidity was greatest, not at a point furthest removed from the surface where there would be least oxygen, but somewhat nearer the surface. All other anaerobic bacteria experimented with showed the same peculiarity, though the level at which the turbidity was greatest was not the same for the different organisms.

This experiment clearly shows that the optimum growth of anaerobic bacteria is secured, not when oxygen is absent altogether, but rather when a very small amount of it is present, the optimum amount being different for the different bacteria. This dictum is applicable to both obligate and facultative anaerobes. Beijerinck also showed that when an anaerobic bacillus is placed in a drop of water the individuals collect, not at a point furthest removed from the surface, but at another point where the oxygen present is such as best suits the needs of that particular bacillus. In consequence of these results he has proposed substituting the words *aerophile* and *microaerophile* for aerobic and anaerobic respectively.

The researches of Chudjakow have also produced important results. He proved beyond question that under atmospheric conditions the oxygen of the air acts directly as a poison to non sporogenous anaerobic bacteria, and if not removed in time its influence causes the death of the anaerobe exposed to it. In the case of a spore-forming culture the spores are not destroyed by oxygen, though they are rendered incapable of germination. But if the amount of oxygen be gradually reduced, a point is reached at which the oxygen not only exerts no harmful effect, but is actually helpful. This applies to *all* anaerobes, they thrive better when a little oxygen is present than when this gas is altogether excluded; and when growing under such a condition, all the oxygen is used up just as it is by aerobic organisms. Before this can take place, however, the oxygen must be present in a very dilute condition. Thus for Bactridium butyricum the amount of oxygen supplied to it must not be greater than what corresponds to 5 mm. of air-pressure. But an amount of oxygen equal to 10 or 15 mm. of air-pressure acts injuriously on this organism, and ultimately results in its death. Similar results are obtained with other anaerobes. Of course, the maximum amount of oxygen that any individual anaerobe can tolerate without injury varies in each case. Whilst for Bactridium butyricum the maximum is 5 mm., for Bacillus oedematis it reaches as high as 25 mm., and for Clostridium butyricum about 10-12 mm. We therefore come to the important conclusion that the *strictest of anaerobes can make use of oxygen provided this gas be supplied in a sufficiently dilute condition.*

In recent years a closer study of the aerobic microorganisms has shown that they also are not unaffected by the amount of oxygen that is present. Thus Bacillus subtilis was rapidly killed when subjected to a pressure of oxygen equal to 10-15 atmospheres. For other organisms, again, very little oxygen suffices for their needs; thus

Aspergillus niger, one of the aerobic higher fungi, can complete its life-cycle when oxygen is present only to the extent of 5 mm. of air-pressure. This last fact is another demonstration of the smallness of the gap that separates the two classes of organisms. Finally, we may add that it is possible both to accustom anaerobic bacteria to a greater and aerobic bacteria to a smaller pressure of oxygen. The breaking down of the sharp line of demarcation between the two classes lends support to the suggestion of Frankland, that anaerobic bacteria have arisen by a process of selection from aerobic organisms; for if we imagine representatives of the latter, in past ages, growing in places where the supply of oxygen was small or where it was rapidly used up, these organisms would either have to give up the struggle or learn to do with less oxygen. In course of time, therefore, the breaking down of organic matter would be accomplished without the aid of oxygen.

As to the mode of decomposition employed by anaerobic bacteria, very little is known, but inasmuch as the products are quite different from those formed by aerobic bacteria, we know that oxygen does not play a part in it. Hence it is improbable that they obtain oxygen from within themselves as a substitute for that of the atmosphere. However, the trend of scientific opinion is in favour of the supposition that anaerobes have found means of breaking down organic matter, and thus securing energy without calling oxygen to their aid, as is done by the whole of the animal world and the vast majority of the members of the vegetable world.

THE SAPROPHYTIC BACTERIA.

The term **saprophyte** is applied to any organism that feeds on *dead* organic matter, whether this be of animal or of vegetable origin. These organisms perform the work of scavengers, for they initiate processes whereby the constituents of the carcases of animals and plants are changed into substances which can serve as food-material to later generations. In this way they prevent cessation of life, for were their activities to cease, all animate nature would soon succumb for want of food. The changes that are accomplished by the saprophytes we term **putrefaction**, and they are usually accompanied by the evolution of foul-smelling odours. If very little odour is manifest, as when decomposition takes place in the open air, it is usual to apply the term **decay** instead of putrefaction, although there is no hard and fast distinction between the two terms.

The organic materials of which the putrefiable matter is composed are split up step by step. Between the original material and the end-products there will, therefore, be a number of intermediate products. Each intermediate product will, generally speaking, be less complex than the one from which it was produced, and in cases where complete decomposition has taken place, the end-products are simple inorganic elements or compounds. Examples of such end-products are, carbon dioxide, hydrogen, mercaptan sulphuretted hydrogen, etc. Further, as will be presently seen, any one saprophyte does not effect all the changes culminating in the formation of the end-products. In each case of completed decomposition a number, usually a large one, of saprophytes will have taken part, in fact, what we call a piece of decomposing matter is in reality the scene of a hard struggle for existence among different kinds of saprophytes in which first one group, then another, gains the ascendency. The struggle ceases either when the food supply gives out, or when a factor is introduced which is equally inimical to all the organisms.

In most cases of decomposition the only organisms concerned are the saprophytic bacteria, though under certain circumstances the higher fungi enter into the struggle, as will be presently shown. In this book we shall be able to deal only with the commonest of the saprophytic bacteria. Some of them are very abundant in the soil and in the atmosphere, and consequently are practically never absent from decomposing matter.

The commonest of all is *Proteus vulgaris* (syn. Bacillus vulgaris, Bacterium vulgare). The morphological characteristics of this organism, and of the group to which it belongs, will be dealt with when we come to consider the sewage-bacteria.

Several colour-producing saprophytic bacteria are well known, and are widely distributed in nature, the best known being *Bac. prodigiosus*, *Bac. fluorescens liquefaciens*, and *Bac. pyocyaneum*.

Bac. prodigiosus was responsible for the "Wunderblut" of the Middle Ages. In 1819, for a whole week, blood-red specks appeared on various articles of food at Padua, and in 1848 there was a similar epidemic in Berlin. However, by the latter time, the science of bacteriology, though still in its infancy, had made some strides, so that when the epidemic appeared in Berlin, Ehrenberg, the famous bacteriologist, had no difficulty in clearing up the mystery of the blood-red specks. He showed that each speck was nothing more than a colony of this species. The individuals constituting the organism are short oval rods, 0·5–1·0μ long. Their shortness gives them the

appearance of cocci, but, as it can be shown that in certain media the individuals grow into somewhat elongated motile rods, the species must be reckoned among the bacilli (Fig. 68). It grows on all kinds of cooked food, particularly during the hot summer months, its growth being easily distinguishable by the red colouring matter which the organism excretes. When grown on potatoes an unmistakeable smell of ammonia and trimethylamine is produced. Very closely allied to Bac. prodigiosus is *Bacterium kiliensi*, which is commonly found on rotting fish.

FIG. 68.—Bac. prodigiosus. Showing rods of different lengths.

Bacillus fluorescens liquefaciens often occurs in abundance in decomposing matter. It is widely distributed in nature, especially in the soil and in polluted water. A culture of this species usually shows a beautiful green fluorescence. The individuals are rod-shaped 1·5–6·0 μ long and 0·4 μ broad. The characteristics of this organism, by which it can be identified, are the following: Green fluorescence, a strongly aerobic nature, an incapacity to form spores, and a power of liquefying gelatine.

Bac. pyocyaneus is another of the chromogenic species which is very commonly found in excrement, in polluted water, and in the soil. On artificial media a growth is produced which possesses a yellowish-green and a blue pigment. This species is suspected by many of being a pathogenic variety of the harmless Bac. fluorescens liquefaciens.

One of the most important of the saprophytic bacteria is *Bacillus coli communis*. It is found as a normal inhabitant of the human intestine and of the intestines of the lower animals. It is consequently always present in dung and various kinds of excrement, and is the commonest of the sewage-bacteria. A full description of this species and its allies will be given in the chapter dealing with the bacteriology of sewage.

All the saprophytic bacteria hitherto mentioned are aerobic, but in addition a large number of anaerobic bacteria play an important rôle in the breaking up of organic matter. The most important of these is *Bacillus putrificus* (Fig. 69), which was discovered in 1899. It is always present in the soil, in decomposing dung, and in liquid manure. Since its discovery it has been isolated from a variety of different decomposing media, so that there is every reason to believe that it has a very wide distribution. The bacilli are 5-6 μ long and 0·8 μ broad,

FIG. 69.—Bac. putrificus.

and the cilia which they bear beyond the average in length. In liquid media long threads are formed and, if cultivated at 30°-10° C., abundant spore-formation is the result. As the spores are usually formed at the ends of the rods, the latter have a characteristic drum-stick appearance (Fig. 70). This species grows well anaerobically, both in media containing and in those devoid of sugar, usually developing an abundance of gas. Gelatine cultures of this species emit a very foul smelling odour.

Fig. 70.—Bac. putrificus. Spore-forming stage.

Other anaerobic bacteria, which are, however, less common, are *Bacillus perfringens*, *Bac. bifermentans sporogenes*, and *Bac. gracilis putidus*. The first two are sporogenous, whilst the last named does not form spores. They differ in the nature of the decompositions that they set up and in some of their morphological characteristics.

The Principal Changes that take place in Decomposition. The decomposition of organic matter does not necessarily always follow along the same lines. The particular course that will be followed depends on the nature of the material, on the amount of sugar that the material contains, on the temperature, on the oxygen supply, on the bacteria that are present, and on several other factors. In fact, it very seldom happens that the details of decomposition are alike in any two particular cases. Yet, in spite of differences of detail, the general trend is very much the same in all, and this trend we now propose to follow. From the bacteriological point of view we may consider all organic remains as consisting of proteids,[1] sugars, and fats in varied proportions.

[1] **Proteids or albuminoids** are complicated substances that occur in animal and in vegetable tissues. Their chemical constitution has not yet been discovered. They contain 54 parts of carbon, 7 of hydrogen, 16 of nitrogen, 21 of oxygen, and 1-1½ of sulphur. They are divided into the following classes :

1. **Albumins**, soluble in water. Ex. *Egg-albumen*.
2. **Globulins**, insoluble in water, soluble in dilute acids and alkalies, soluble in 1 per cent. common salt solution. Exs. *Globulin*, *myosin*.
3. **Derived albumins**, insoluble in water and common salt solution, soluble in dilute acids and alkalies. Ex. *Casein*.
4. **Fibrin**, insoluble in water, sparingly soluble in dilute acids and alkalies and in neutral saline solutions. Exs. *Fibrin* and *gluten*.
5. **Coagulated proteids**, soluble in gastric juice. Ex. *Coagulated albumin*.
6. **Amyloids**, insoluble in gastric juice.

We may divide the bacteria that decompose proteids into two classes. To the first belong those that decompose the naturally occurring proteids into **albumoses**[1] and **peptones**,[1] whilst in the second are included those that attack these albumoses and peptones, decomposing them into **amino-acids**. The amino-acids are then further decomposed by various kinds of bacteria.

The first class includes all the anaerobic bacteria that are saprophytic (except Diplococcus magnus aerobius), Proteus vulgaris, Bac. fluorescens liquefaciens, and Micrococcus pyogenes, whilst examples of the second class are Bac. coli communis and Bac. prodigiosus.

The amount and nature of the sugar content of a decomposing mass are the chief factors which determine the course of decomposition. The reason for this lies in the fact that bacteria change the sugars into various acids, which very materially affect the growth of the various competing bacteria. For whilst some are prevented from developing or are even destroyed by the presence of a certain quantity of acid, others are more resistant and are not hindered in their growth by the presence of the same amount of the same acid. The more resistant organisms obviously benefit by the weakness of the others.

Whilst some of the saprophytic bacteria decompose only the proteids, others attack only the sugars, and a third group attacks both the proteids and the sugars.

As explained, a mass of putrefying matter may be regarded as an arena on which a number of bacteria are constantly struggling to obtain the food contained in the matter. But the conditions which make for success are constantly changing, because new substances are continuously being produced by the activities of the successful bacteria, which are generally unfavourable to their welfare. Hence they disappear or become very much reduced in number, and a new set of bacteria become masters of the situation, only to be in their turn replaced by other competitors as further changes take place. All the changes that take place in the organic matter are in one direction, they consist, namely, in a breaking down of the complex proteids and other materials into simpler substances, and stop either when substances are at last produced which are unsuitable for bacteria to feed on, or when the percentage of acids formed from the sugars is great enough to make bacterial life impossible.

[1] **Albumoses and peptones** are derived from albumins. They possess the same chemical structure and can be classed as albumins. They differ, however, from the substances from which they are formed in being more soluble, and in possessing different physical properties.

In the case of organisms like Bac. coli communis and Proteus vulgare that attack both proteids and sugars, the proteid-decomposition is hindered very much if much sugar be present in the medium. In such a case, the usual result is a temporary cessation of bacterial activity owing to the production of acids, into which the sugars are transformed. The decomposing material, however, forms now a suitable medium for the growth of moulds and allied fungi, if the supply of oxygen be sufficient. The result of the activity of such organisms, is a still further decomposition of the organic matter, and at the same time a total or a partial removal of the acids. This removal gives the bacteria another chance, with the result that competition among the different kinds once more sets in, and the proteid matter is still further decomposed. This goes on until either unsuitable products are formed or until the amount of acid is once more sufficiently large to prevent further bacterial growth. In the latter case the moulds and other higher fungi will again step in and be again ousted by the bacteria when the acidity has been removed.

If decomposition takes place in the absence of an adequate supply of oxygen, the products that are formed are of an entirely different character to those that are formed when the supply of oxygen is plentiful. The proteids are not so completely broken up, and such substances as *skatol*, *indol*, *sulphuretted hydrogen*, and other foul-smelling substances are produced. A good example of such decomposition is that which takes place inside the intestines where anaerobic conditions hold.

With regard to the fats contained in putrefying matter, they need not be considered here, as they do not affect the course of putrefaction. We shall obtain a good idea of the course of the changes that take place if we take concrete instances and follow in them the stages of decomposition so far as this is possible.

(*a*) **Putrefaction of Meat.** The aerobic bacteria, which decompose both proteids and sugars, are the first to start decomposition in meat. As there is only about one per cent. of sugar in meat, the production of acids is not great, so that the proteid-decomposition is not appreciably hindered owing to their presence. The organisms that appear first are Proteus vulgaris, Bacillus coli communis, and certain members of the Cocaceae, *e.g.* Micrococcus pyogenes. These predominate for a time, during which a large amount of proteids is broken down and a very small amount of acidity developed. The smallness of the acidity is to be accounted for, not only by the smallness of the sugar-content in meat, but also by the neutralisation

of the acids which is effected by the ammonia, that is freely developed during this kind of decomposition. After three or four days some anaerobic bacteria begin to predominate, especially Bac. perfringens, and Bac. bifermentans sporogenes. These use up both the proteids and the sugars and after about eight or ten days, all the sugars have disappeared. The removal of the sugars results in a predominance of anaerobic bacteria, which decompose only the proteids, such as Bac. putrificans, Bac. putidus gracilis and Diplococcus magnus anaerobius. These anaerobes usually predominate until all the proteids are used up. The greater part of meat-decomposition is therefore performed by anaerobic bacteria. The initial predominance of the aerobic bacteria results in the removal of oxygen from parts near the surface, and owing to the close texture of the meat, there is very little free oxygen in the deeper layers.

(*b*) **The Decomposition of Milk.** As milk contains about four per cent. of the sugar *lactose*, the course of development is essentially different to that which takes place in meat. The first organisms to predominate are usually Bac. subtilis and its aerobic allies which decompose proteids, also organisms like Bac. coli communis and Streptococcus acidi lactici that ferment both proteids and lactose. In a very short time, however, the milk is completely monopolised by the lactic-acid bacteria which are able to multiply in this medium at an enormous rate. They transform so much lactose into lactic acid that all bacterial development is temporarily arrested. Before this happens, however, a portion of the proteids contained in milk will have been decomposed by them. If now no air be permitted to enter, or if its supply be inadequate, no further changes take place. But if air be freely allowed to enter, as for example, when milk is exposed in open pans, some of the higher fungi, especially Oidium lactis appear in the milk after a few days' exposure. These split up a portion of the remaining proteids, and also decompose the lactic acid. The latter process diminishes the acidity of the milk, and after a few more days the lactic-acid bacteria are again able to multiply. The same process is now repeated, so that some more of the proteids and some more of the lactic acid are decomposed. This alternating preponderance of the bacteria and the higher fungi, goes on until the proteids and the sugar are almost completely used up. Sometimes there may be at the same time a subsidiary fermentation, set up by the butyric-acid bacteria, which change the lactose into butyric and propionic acids. These substances, when present, impart a very disagreeable

smell to the decomposing milk. This fermentation, however, never becomes very great, owing to the severely restraining influence on it, of the lactic acid.

As the higher fungi preponderate only so long as the acidity is high, the bulk of the decomposition is performed by the lactic-acid bacteria. After two or three months, organisms like Proteus vulgaris make their appearance, and complete the decomposition Putrefactive bacteria in the strict sense of the term have very little to do with the decomposition of milk, as the lactic-acid bacteria cannot be included under this class of bacteria.

(*c*) **Eggs.** The outer covering of eggs is not sufficient to ward off bacterial attacks, as bacteria find no great difficulty in penetrating the shell. Further, bacteria are present even before the shell has been deposited, and consequently even a fresh-laid egg is never free from bacteria. The decomposition of an egg takes place along one of two lines. In the first, the albumen changes into a whitish-gray or greenish-gray colour, whilst the yolk at the same time gradually turns into a blackish-green slimy mass, which later fuses with the decomposed albumen, the whole forming a pulpy mass which smells very strongly of sulphuretted hydrogen. From this mass one investigator has isolated ten species, all of which have been temporarily included under one name, viz. *Bacillus oogenes hydrosulphureus.* In the second mode of decomposition, no sulphuretted hydrogen is formed. Both the yolk and the albumen are changed into thin liquids, which later become pulpy and have the same smell as human excrement. The cause of this change has been ascribed to five species of bacteria, all of which have been termed *Bacillus oogenes fluorescens.* All five are strongly aerobic, and as the shell is penetrable to gases, there is no difficulty in securing an abundant supply of oxygen from the atmosphere. The methods for the preservation of eggs will be described in the chapter dealing with the preservation of food-products.

We cannot in this book enter into a detailed description of the various intermediate substances that are produced in any mass of decomposing matter. The amino-acids, produced by the breaking up of peptones and albumoses, are the first substances to be formed, the chemical constitutions of which are known. From the production of amino-acids to the formation of the end-products almost all the principal changes can be expressed as chemical equations. The number of intermediate products thus formed is very numerous. Amongst them *Indol* is to be reckoned. This is a foul-smelling substance produced by a number of bacteria in nutrient media containing peptone. As it

forms a red compound with nitrous acid, its presence in a nutrient solution is easy to identify. This fact is made use of in the diagnosis of those species that are known to produce indol. *Vibrio cholerae*, the dreaded cholera organism, produces much indol, and it was in cultures of this organism that the reaction was first observed. It is often in consequence called the *Cholera-red reaction*. Indol is also produced by Bac. coli communis, a fact which is made use of, to distinguish this organism, from Bac. typhosus, which it resembles in many respects. Closely allied to indol, and smelling even worse, is *Skatol*. This substance is formed towards the end of putrefaction, and together with indol is responsible for the unpleasant smell of excrement. The sulphur that is present in proteids appears in the end-products chiefly as sulphuretted hydrogen or as methyl mercaptan. Other inorganic end-products are marsh gas, free hydrogen, carbonic acid, free nitrogen and ammonia, all of which are given off into the atmosphere in the form of gases. Amongst the products of the decomposition of proteids, mention must be made of the *ptomaines* that are produced from them. These form a special class of organic substances, all containing nitrogen, and having a high molecular constitution as well as possessing a number of physical properties in common, *e.g.* solubility in hot alcohol, bitter taste, etc. A large number of these ptomaines have been found, some of which are harmless and others very poisonous. Examples are *Neuridin* ($C_5H_{14}N_2$), *Trimethylamine* (C_3H_9N) and *Cadaverin* ($C_5H_{14}N_2$), which are poisonous only in large quantities. On the other hand some are extremely poisonous.

Observations on Pure Cultures of Putrefactive Bacteria. By observing the changes that take place in nutrient media, in which a pure culture of one or other of the putrefactive bacteria is present, it is possible to ascertain to some extent the changes produced by the putrefactive bacteria in nature. This is indicated by the following table:

Organism	Acting on	Produces
Proteus vulgaris	Gluten and fibrin	Phenol, Indol, Amines and Fatty acids.
,,	Casein	Albumoses, Peptone and Amino-acids.
Streptococcus longus (anaerobic)	Fibrin	Tyrosin, Leucin, Amines and Fatty acids.
Bacillus coli communis	Casein	Albumoses.
,, ,,	Peptone	Ammonia and Indol.
,, ,,	Mixture of eggs and flesh	Skatol, Phenol, Aromatic Oxy-acids, Leucin, etc.

Organism	Acting on	Produces
Micrococcus pyogenes	Gluten	Phenol, Indol, Amines and Fatty acids.
Aerobic peptonising lactic-acid bacteria	Casein	Leucin, Tyrosin, Fatty acids, Aromatic Oxy-acids and Tryptophan.
Bacillus subtilis and Bac. prodigiosus	Albumoses	Leucin, Tyrosin and Tryptophan.
Vibrio cholerae	Albumen	Leucin, Tyrosin, Indol, Amines, and Fatty acids.

These and similar results cannot give all the changes that take place even in pure cultures, but only those involving the production of substances that chemists can identify. Again, in nature the conditions are different owing to the presence of different kinds of bacteria on the decomposing material, which cannot but influence not only the amount of, but also the nature of, the decomposition of any one of the competing organisms.

CHAPTER IX.

§ 1. PATHOGENIC BACTERIA.

Introduction. It is surprising that so much misconception should exist as to the functions of bacteria. To the minds of the vast majority of even educated people the term "bacteria" is synonymous with "small organisms that breed disease." They overlook, or rather are not aware of the fact, that the number of bacterial species which are pathogenic is small in comparison with the large number which not only do good but fulfil functions upon which our very existence depends. There are, however, out of the total of nearly two thousand known species a few, some thirty species in all, which, from the purely human standpoint, must be regarded as scourges, though possibly if considered from the wider standpoint of the economy of nature they would not be looked on in this light. In this chapter we propose to deal with the broad facts connected with the activities of this class of bacteria.

All pathogenic bacteria cause a disturbance in the human system, if they are able to multiply. The disturbance is caused by poisons secreted by these bacteria, and this secretion does not take place except when the bacteria are multiplying. Hence the mere presence of pathogenic bacteria is not in itself harmful. Thus, many millions of cholera germs might be present in the body without any harm accruing to the individual: the harm commences only when these begin to multiply. The poisonous secretions of these bacteria are called *toxins*, and all diseases due to bacteria may be regarded as poisoning processes. An inoculation of a toxin produces the same effect on an animal, as is produced by the inoculation of the bacteria which produce that toxin. The toxin-inoculation is however more rapid in its effects, as is to be expected, for it takes some time for the bacteria to produce the poison in quantity. Such being the case, we can readily under-

stand the distinction which is made between the two classes of pathogenic bacteria. Bacteria may, for example, feed on cheese and secrete a poison as one of the products of decomposition and then die off. Their poisons will be present in the cheese. If a person now eats a portion of the cheese he suffers from the effects of the poison, although not a single live microbe of the species that produced that poison enters his body. This is different to the case of the person who eats a portion of cheese which contains no poison, but a few microbes of a kind that is able to grow and multiply inside that person's body. In this case the poison is manufactured inside the body, the microbe being the parasite and the person the host.

§ 2. BACTERIAL DISEASES OF THE ANIMAL KINGDOM.

A large proportion of our food consists of substances upon which bacteria as well as ourselves find nourishment. If a poison-secreting microbe multiplies in food, and the latter is afterwards consumed, the consumer suffers from the effects of the poison produced by the microbe. In this way poisoning from eating decayed meat, cheese, ice-cream, fish, etc., is caused, and the effect is generally rapid, though if the bacteria are not alive when the bad food is eaten, recovery is rapid so soon as the body throws off the poison, for there will be no bacteria to produce more of it. In warm weather the danger from this source is greater than in cold weather, because the temperature favours a rapid multiplication of these organisms, and this is especially so in the case of milk. The most important disease of this kind is the *Cholera infantum*, which is common among infants who are nourished entirely on cow's milk. We have a good instance of the other kind of pathogenic bacteria in Bacillus typhosus, which is the cause of typhoid fever. This microbe grows and multiplies in the intestines, and whilst not invading the body generally, becomes localised in special glands like the liver and spleen. The poison which is secreted is called *typhotoxine*. In the case of *diphtheria* we have an instance of bacteria that cannot be strictly included in either of the two classes of pathogenic bacteria, for though **Bacillus diphtheriae** is found in the mouth and throat of patients suffering from this disease, it is found only in the false membrane of these parts. They do not enter into the deeper tissues of the body, though they grow and multiply in the false membrane, secreting a poison which causes the disease. The bacillus is composed of slender rods, about 3 μ in length, and sometimes slightly

curved. When stained with methylene-blue the bacilli assume a deep blue colour, and inside them granules of a still deeper colour are also visible. The latter give the rods a characteristic beaded appearance. Occasionally, also, the ends of the rods are swollen after staining, sometimes so much so that a club-shaped form is assumed.

When a cut is made in the skin by an object which is contaminated with earth or dung, a germ called **Bacillus tetani** sometimes gains entrance into the body and gives rise to the disease called *Lock-jaw*. The bacteria are localised in the neighbourhood of the wound, but the poisons secreted by them infect the whole body. The rods are usually 4-5 μ long and rather narrow, being only about ·4 μ in breadth (Fig. 71)

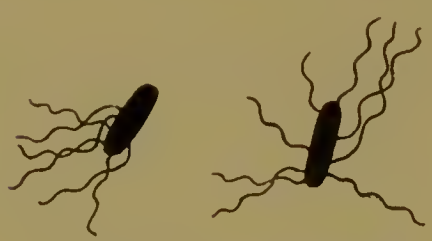


Fig. 71.—Bac. tetani. Showing cilia.

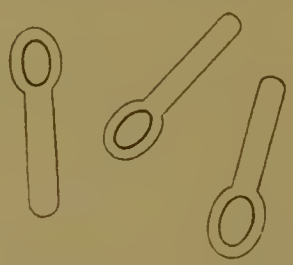


Fig. 72.—Bac tetani. Showing spores.

They form spores readily, which are usually situated at one end of the rod, giving the latter a very characteristic drum-stick appearance (Fig. 72). The toxin of this bacillus is extremely powerful, as even 0·0005 milligramme of an impure preparation has been found sufficient to kill a mouse. The wound from the contaminated object may be very small, in fact, may consist of a mere abrasion of the skin, but if these bacteria once get settled on that point it is not long before the whole body is poisoned. This disease is most prevalent among people who work in byres, in farmyards and on the soil, where the bacillus is most plentiful. Fortunately, though abundant, the bacillus only rarely gets a hold, because before full effect can be produced there must be either symbiosis with another organism, such as Staphylococcus pyogenes aureus, or else some stimulating cause in the shape of a mechanical irritant. The irritants in this case are the presence of lactic acid, or the presence of soil or small splinters of wood in the wound.

A much dreaded parasite is the "comma bacillus" or the "cholera spirillum." The individuals of this germ are not rod- but comma-shaped (Fig. 73). Here, again, the individuals are found only in one part of the body, whilst the poison secreted by them permeates the whole system. They are normally motile, and measure each about

1·5-2 μ in length and about 0·4 μ in breadth. The "commas" sometimes become attached and thus form a combination, something similar to the letter S in shape. This organism is found, during the disease, only in the intestines: it does not enter into the blood or attack any of the other internal organs. It also thrives as a saprophyte in various kinds of decomposing matters, though very little is known of its saprophytic habit. The conditions promoting its growth are a fairly high temperature, a plentiful supply of oxygen and of organic material. This microbe is easily killed, being very susceptible to drying. Hence we do not find that cholera epidemics spread very far from their starting points, unless the microbe is carried by ships or by other means of transit. The spread of this disease is almost always due to the access of choleraic discharges to the water supply. As flies fed on material containing the cholera microbe can retain the latter in a live condition for twenty-four hours, it is probable that insects are also instrumental in serving as vehicles for the transmission of this disease.

Fig. 73.—Cholera spirillum.

Very little is known of the poison secreted by the cholera microbe. It has been separated, but only in an impure condition. When animals are inoculated with it all the symptoms of cholera rapidly appear, even though the organism itself which produced the poison has been killed. Since Koch's researches (published in 1884) the cholera germ has been extensively studied, and as the cause of the disease is known, the methods of combating this scourge have attained a high degree of efficiency.

Another dreaded parasite is **Bacillus tuberculosis**, which also confines its growth to limited areas. This microbe is widely distributed, and is responsible for consumption, "white swelling" of joints, lupus, scrofula, etc. It may attack almost any organ in the body; thus it may be found only in a small spot in the lungs, or in one joint or in one gland; but, on the other hand, invasion may take place from these spots and several organs become infected. This invasion may be slow or rapid, and if persistent will end fatally. The discovery of this germ is due to Koch, whose research on this subject is one of the masterpieces of bacteriological science, on account of the enormous difficulties which he successfully overcame. Bac. tuberculosis attacks domestic animals with deadlier effect than any other microbe. It is

commonest among cattle, but is also quite common among pigs, horses, dogs, cats, and fowls. Sheep and goats appear to be immune to this disease. Whether the species causing tuberculosis in the human subject is absolutely identical with that causing the same disease in cattle is still undecided, for though Koch maintained at the Tubercular Congress of 1901 that they were quite distinct species, the general opinion of the medical world is against him, maintaining that the same species causes the disease both in man and in cattle. The subject is still under investigation. Its importance is obvious when we reflect on the quantity of food in the shape of milk that we obtain from cows.

With regard to avian tuberculosis, there is reason to believe that the human subject is not susceptible to the bacillus obtained from a tubercular fowl, as the following results show:

1. Human tubercle bacilli produce no effect when a solution containing them is injected into birds.
2. Human tubercle bacilli produce acute tuberculosis when introduced in the same way into dogs.
3. Avian tubercle bacilli produce acute effects on birds but not on dogs.

It seems, therefore, extremely probable that the avian Bac. tuberculosis is a different variety to the kind found on man and dogs, though of course belonging to the same species. Unfortunately, however, there is every reason to believe that the avian is only a modification of the human variety, for by gradually accustoming the latter to the conditions under which the avian variety usually grows, it can be induced to assume all the characteristics of the avian variety. The rods of Bacillus tuberculosis are very small, being only 2·5-3·5 μ long and ·3 μ broad (Fig. 74), though sometimes they are longer—up to 5 μ or more. They are usually straight, but also sometimes slightly curved. These bacilli take up stains very slowly, so special methods must be employed for colouring them. When they have taken up a stain they retain it tenaciously: thus, whilst a 20 per cent. solution of sulphuric acid immediately takes away the colour from most stained bacteria, in this case the decolorising agent must be allowed to act a long time before the colour is removed. Hence, in a mixture of tubercle bacilli and other bacteria it is possible to make a preparation in which the former only are stained. The tubercle bacillus, however, is not the

Fig. 74.—Bac. tuberculosis.

only species that exhibits this peculiarity with regard to stains, though the number of such organisms is not great. They are classed together as "acid-fast bacteria." Much research has been undertaken to find out the peculiarities of growth, etc, of the acid-fast bacteria, in order to be able to distinguish them readily from Bac. tuberculosis, so as to facilitate the diagnosis of the latter. Most of these bacteria have been obtained from sources outside the body, hence, if the material under observation has been derived from the latter source, and yields an acid-fast bacillus, the probability of this microbe being Bac. tuberculosis is very great.

The animal body is the natural feeding-ground of this germ, in which alone it can multiply. Infection takes place almost exclusively through the air. The sputum of a consumptive patient contains large numbers of these bacteria, and when the sputum dries up they are conveyed by the atmosphere to other places. The resistance power of the bacilli is very great. In a dry condition they are virulent even after two months' exposure to the air, and even in a moist condition their vitality is not impaired for several weeks. Hence, whether in the dry or in the moist condition, the danger from these germs is not over for a long time after their liberation from the body of a tuberculous patient. Another point worth noticing is that these bacilli have been found in a live condition in tubercular organs many weeks after the latter have been buried. It is not surprising, therefore, that the bacillus of consumption does not visit us in the form of an epidemic, but rather attacks one here, another there, according as it finds a suitable medium for its growth and multiplication. The germs are present in most places, but are naturally in greater number in crowded towns.

The poison secreted by Bac. tuberculosis is not well known, but its existence is made evident by the effect that Koch's *tuberculin* has on human subjects. Tuberculin is a concentrated glycerin-bouillon-culture of Bac. tuberculosis. After sterilisation of this culture it was found that 0·25 c.c. of the liquid caused a healthy man, after three or four hours, to suffer from malaise, laboured breathing and a tendency to cough, all of which, however, passed off after 24 hours. Tuberculin was introduced by Koch as a curative agent on the same principle as vaccination for the prevention of small-pox. Unfortunately, it has not been successful, neither can the same be said of the new tuberculin which was introduced by him in 1897.

To this group must also be reckoned **Bac. anthracis**, which fortunately does not often attack man. The disease is known as *Anthrax* or *Malignant Pustule*. The bacillus multiplies rapidly in the blood,

causing an infection of the whole body, generally with fatal results. The bacilli are found everywhere in the body of the infected subject. The disease occurs as an epidemic among herbivora, and though not a natural affection in man, may be passed over to him from animals either directly or indirectly. A drop of blood taken from a cow which has died of anthrax will show a number of non-motile large rods (Fig. 75). The ends of the rods are somewhat rectangular, not gently rounded off as are most bacterial rods. Each rod is about 1·2 μ in width and about 6 μ in length, so that its size is above the average.

Fig. 75.—Bac. anthracis.

The cultures of this species on gelatine- and agar-plates, in stab-tubes, etc., have such well-defined characteristics, that, taken with its microscopical characteristics, the species is not difficult to identify. Unlike those which we have previously dealt with, this species forms spores abundantly, hence their resistance to heat and antiseptics is considerable, being much greater than that of any of the other pathogenic bacteria.

This bacillus finds a ready host in cattle, sheep, guinea-pigs, mice and white rats. On the contrary, flesh-eating animals (dogs, cats, etc.) are practically immune, and the same can be said of birds and of animals belonging to the same group as the frog. Man occupies an intermediate position, so far as susceptibility to this disease is concerned, between the highly susceptible and the highly immune creatures. This disease usually appears among those who habitually handle the skins or the carcases of animals. The spores of diseased animals enter the human system through cuts or abrasions of the skin or through the hair follicles, or perhaps by being inhaled, for highly susceptible animals can be infected by being made to inhale the germs. Once inside the body the microbe spreads everywhere, and its toxins are secreted throughout the whole body.

Now, with regard to the mode in which these bacteria are spread, an animal suffering from anthrax sheds into the air by bloody excretions from the bowels, mouth and nose a large number of microbes belonging to Bac. anthracis. These will probably form spores if they do not at once find a nutritive medium. In the spore condition they can remain alive for years. If they come in contact with a favourable medium the spores germinate, and in this way many millions more are produced. As this microbe is not only parasitic on animals, but also saprophytic on various organic matters in the soil, its chances of continued existence are very considerable. Naturally, also, the carcases

of animals that have died of this disease are full of them, so that such carcases also spread the disease, unless treated to prevent further infection. It has, however, been found that in the process of putrefaction the anthrax bacilli succumb rapidly when in competition with the putrefactive bacteria.

The disease known as *Glanders* is commonest among horses and allied animals, *e.g.* mules and asses. In man it is the result of direct inoculation from an animal through a skin wound, so that the disease is chiefly found among people who have much to do with horses. The organism which is responsible for this disease is named **Bacillus mallei**, and consists of short rods with rounded ends of about the same size as those of Bac. tuberculosis. They are about 0·3 μ in breadth and about 3·0 μ in length. Sometimes filamentous rods 8-12 μ long are to be found in a culture of this bacillus. Spore formation is unknown. The bacilli can retain their vitality for 14 days, sometimes longer, when not interfered with, but are easily killed by heat and disinfectants. Glanders is very like tuberculosis in its action and general character. It spreads from a diseased animal by direct contagion from the nose or from sores. So far as is at present known infection through the medium of air to man does not take place. The poison secreted by this organism has been prepared in an impure condition, and is known as *mallein*. This is used in inoculation of animals for preventive purposes, the poison being administered in such a way that a mild attack of the disease takes place. This renders the subject immune so far as this disease is concerned. Another disease organism belonging to the same group of bacteria is **Bacillus influenzeae**. During an attack of influenza the bacilli are found in large numbers in the nasal secretion, and in still larger numbers in the masses of greenish-yellow sputum from the bronchi, where they occur almost in a state of purity. They are small, being only 0·3 μ broad and not more than 1·5 μ long (Fig. 76). We do not know much of this bacillus, and the chief evidence of its casual relation to the disease is based on the fact that it is always present in these nasal and bronchial secretions during an attack of influenza. Like tetanus and tuberculosis, the bacillus is confined to certain areas, from which doubtless the toxin is distributed to other portions of the body. Much information cannot be obtained, because, so far as is known, all the lower animals are immune against this disease. With regard to the contagiousness of influenza, it is known that the resistance of

Fig. 76.—Influenza bacillus.

the bacilli is very small, for 24 hours' exposure to the atmosphere is enough to kill them, and even in water containing no source of nutriment two days were found to be sufficient to complete their destruction. Contagion must therefore ensue, if at all, within a day after the bacteria have left the body. This is effected by means of the spitting, coughing and even speaking of the infected persons, whereby small particles of the mucus are ejected. The reason why influenza is more "catching" than most other diseases is owing to the fact that the bacilli may remain alive inside the body for weeks, if not months, after the patient has recovered from an attack. This is shown by the fact that it is possible to make a culture of Bac. influenza from the sputum of a person several weeks after recovery from an attack of this disease.

Gonorrhœa is a disease associated with **Micrococcus gonorrhœae.** The organism consists of round cells. Micrococcus is the generic name for cocci, which divide in two directions of space. This micrococcus is usually found in couples (Fig. 77). In the pus of acute gonorrhœa many micrococci are found both in the male and in the female. In the earliest stage of the disease a considerable number of the micrococci are found either free or else adhering only to the surface of the desquamated epithelial cells, while in later or more acute forms of the disease there is a greater penetration into the tissues. The micrococci are only locally distributed. The toxin of this organism has been prepared by growing the organism in an appropriate medium for twelve days. When inoculated into the human urethra, either male or female, this toxin produces all the symptoms of the disease, even though the cocci themselves are not present. Inoculation of the toxin into the bodies of lower animals seems to have no effect on them.

Fig. 77.—Micrococcus gonorrhœae.

In the peculiar disease called *leprosy*, a microbe called **Bacillus leprae** is always present, but, hitherto, all attempts to cultivate it outside the human body or the lower animals have failed. It occurs in certain parts of Europe—Norway, Russia, Greece, etc.—is very common in parts of Asia, *e.g.* Syria and Persia, and is also found in Africa, along the coast, among the Pacific Islands, and in the warmer parts of North and South America. It is a chronic disease, a great amount of tissue change being produced, with very little detriment to the general health. The bacillus is a thin rod, about the same size as the tubercle bacillus, which it also resembles in

other respects, viz. in being slightly curved and in its staining characteristics. The disease is, fortunately, not easily incurred by the human subject, and not at all by the lower animals. A criminal in the Sandwich Islands was inoculated in several parts of his body with leprous tissue, and died from its effect several years later, but in several other experiments negative results only have been obtained. The method by which the disease is carried from one person to another is still a controversial matter. Some maintain that it is hereditary, but it is known that leprous subjects can bear children free from the disease. On the other hand, the case of Father Damien, who contracted the disease after going to the Sandwich Islands, shows that it must be partly infectious. We may, therefore, conclude that whilst the disease is infectious, its contagiousness is of a low order, and it is probably hereditary, only to this extent, that some people are more liable than others to catch the disease, owing to the peculiarity of their organisation, which renders their bodies a good nutritive medium for these bacteria.

We come now to a bacterial disease caused by an organism which belongs to the Spirillaceae. This is **Spirillum Obermeieri**, or **Spirochaete Obermeieri**, as it should more properly be called. The disease for which it is responsible is called Relapsing Fever. As seen in Fig. 78, this organism is like a corkscrew in shape. In the blood, during fever, the spiral may be from two to six times the diameter of a blood corpuscle. Obermeier found that the microbe disappeared about the time of the crisis of the fever, and reappeared again when a relapse occurred. The disease has been experimentally communicated by inoculation to man and to apes. The spirals are actively motile, and move in the manner peculiar to organisms of this kind, namely, a forward accompanied by an undulatory movement. The individuals of this organism are found throughout the whole body, and are not confined to local areas. A cultivation in a pure state has not yet been achieved, so that very little, either of it or of its toxin, is at present known.

Fig. 78.—Spirillum Obermeieri.

The term Pneumonia is applied to several kinds of illnesses, but all are varieties of lung inflammations, in which modifications are produced depending on the special structure of the lung or of the parts of it which are affected. Two bacteria are associated with this disease, viz. **Fraenkel's pneumococcus** and **Friedländer's pneumobacillus.**

Fraenkel's pneumococcus is a small oval coccus about 1 μ across its longer side. The cocci are generally in pairs (Fig. 79), but sometimes there may be 4-10 cocci attached together. The term "pneumococcus" was a temporary one, and it is now usual to replace it by the generic name "micrococcus," to which group the organism belongs. In true croupous pneumonia this species is by far the most frequent organism that is present. The free ends of a group of attached cocci are often pointed in the form of a lancet. Each group has usually a transparent covering enveloping and protecting it. This covering or *capsule* forms a kind of halo round the group of cocci.

Fig. 79.—Fraenkel's pneumococcus.

Friedländer's Pneumobacillus is rod-shaped with blunt rounded ends. It agrees with Fraenkel's pneumococcus in its capsule formation and in the tendency of its individuals to form pairs. Its occurrence is, however, much rarer, being present in only about 5 per cent. of the cases of pneumonia, in fact, its causative connection with this disease is very doubtful.

Fraenkel's pneumococcus, which has an undoubted causal connection, has been isolated and closely investigated. The name *pneumotoxin* has been given to the poison secreted by this microbe. When a patient succumbs to this disease, the immediate cause of death is heart failure and general nervous depression, resulting from the pneumotoxin, rather than suffocation resulting from the interference with the functions of the lungs. Animals can be rendered immune against this microbe by being inoculated with a mild form of the toxin.

In addition to the species that have been described, many others are known, which have some connection with one or other of the various diseases with which we are inflicted. In most of these cases, however, very little is known of causative organisms, and their power of bringing about disease is dependent in many cases on the co-ordination of other bacteria, that is, there must be symbiosis between two or more organisms. This makes investigation more difficult. Again, many of the less harmful diseases are due to the toxins secreted by **Staphylococci**. These organisms are found associated with abscesses, pustules on the skin, carbuncles, boils, and also some catarrhs and ulcers. The best known of these bacteria is Staphylococcus pyogenes aureus (Fig. 80). This

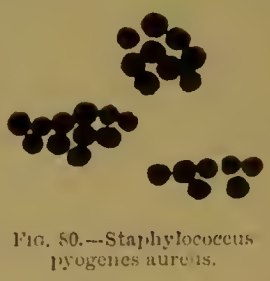


Fig. 80.—Staphylococcus pyogenes aureus.

coccus is about 0·9 μ in diameter and grows in clusters. Another is Staphylococcus pyogenes albus, which exhibits a white growth in all nutrient media. Some of these Staphylococci have much vitality, thus, S. pyogenes aureus can stand half an hour's exposure at 80° C. without injury.

We have dealt with the better known of the pathogenic bacteria that attack man and the lower animals. Extended research will probably show that other diseases, *e.g.* bronchitis, whooping-cough, and measles, are also due to poisons secreted by pathogenic bacteria that have found an abiding place inside our bodies.

§ 3. BACTERIAL DISEASES OF THE VEGETABLE KINGDOM.

In speaking of pathogenic bacteria, we must not forget that under this term must be included those organisms that ravage the vegetable as well as those that ravage the animal kingdom. The general principles underlying the liability to disease are the same. If a plant be injured, the point of injury is a weak spot in its defences, through which the investing bacteria or moulds, which are always present, are sure to effect an entrance unless the plant can cover up the weak spot by means of a layer of cork or by some other way. In the case of plant diseases, moulds are nearly always the originators. In some cases, they act in conjunction or in competition with bacteria, and in still fewer cases, bacteria only are found. The vine-disease known as **Mal vero** is an instance in which only bacteria are responsible. Black spots and streaks appear on the leaves of the vine. At first they were supposed to be due to the tannin, a good deal of which is found in this plant, but now it is known that these spots and streaks are due to bacterial action. Again, there is very little doubt that the **cankering** of trees is in large part due to bacteria. Cankers are irregular excrescences due to the perennial struggle between plant tissues which are attempting to heal up a wound, and some organism or other, which is striving to keep the lesion open. The wound must be one which extends to the cambium of the tree, for this tissue is the only part inside the matured stem capable of forming new plant cells. Insects are usually responsible for the infliction of the wound, which is of the nature of a deep puncture in the wood. Very little is known of those plant diseases in which both bacteria and moulds are found together. Cases of symbiosis between these organisms are known to occur when disease follows the infliction of wounds on the

rose, ash, and olive trees. Again, in the case of those plant diseases that are characterised by exudations and by rotting of the plant, it is very probable that bacteria are partly or wholly responsible, for there are multitudes of bacteria in the putrefying mass. Up to the present, however, no casual relation has been established, for more than the mere presence of an organism in a diseased part is necessary to prove that the disease is caused by this organism.

A familiar plant disease is the rot of onion. This is caused either by a fungus called **Botrytis**, or by one called **Sclerotinia**. These are followed by moulds, yeasts, and bacteria, which complete the work of destruction, though they would be ineffective were it not for the inroad previously made by Botrytis or Sclerotinia.

A similar case can be instanced in the "leaf-curl" of the potato plant. The leaves become flaccid and yellow, whilst the stem, just above the soil, droops and blackens. This is caused by a white mould, but the weakened plant is attacked by bacteria, which cause the tubers to peel in shreds, the flesh to become more or less pulpy, and to emit a nasty smell. The chief agent in this secondary work of destruction is an anaerobic bacillus of the kind called **Clostridium**. (Fig. 81). This microbe so thoroughly decomposes the tuber that the latter becomes a mere bag filled with loose starch grains and a foul-smelling liquid.

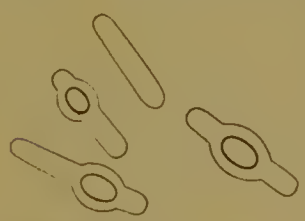


Fig. 81.—Clostridium form of bacillus.

The dry-rot of potatoes is due to the mixed action of various bacteria and fungi. In this case the work of destruction is very slow. The cell-walls of the potato are slowly destroyed by a bacillus of the clostridium form: the destroyed tissues turn brown, the whole tuber being finally reduced to a shrunken mass of a crumbly consistency.

Of late years successful attempts have been made to investigate plant diseases, on the lines followed by the investigations of animal diseases. A good example is supplied us by Potter's investigation of a bacterial disease which afflicts the turnip plant, and to which the name "White Rot" has been given. The leaves droop and become yellow in colour, the roots turn soft, and ultimately the whole plant is destroyed. The organism responsible for this disease is **Pseudomonas destructans**. It consists of short rods, each with one cilium at one of the ends. The isolation of this microbe, and its causal connection with the disease in question, have both been successfully accomplished. The toxin which is secreted in this case

is *oxalic acid*. It was found that the inoculation of the plants with this acid produced the same effects as their inoculation with the bacteria. The destruction of plant tissues by such organisms is effected by the secretion of ferments, some of which change the cell-walls into a pulpy mass, whilst others change insoluble into soluble and more digestible substances. Three of these ferments are known to be secreted by Pseudomonas destructans. One called *cytase* softens the cell-wall, causing it at the same time to swell up, and also destroys the middle lamella. Another called *diastase* changes starch into sugar, whilst the third is one which must be able to decompose proteids, for in gelatine cultures of the organism, liquefaction of the gelatine takes place. These bacteria gain an entrance into the turnip through wounds caused by snails, slugs, larvae, etc., which bore holes or feed upon certain portions of the plant. The secretion of oxalic acid is a common phenomenon in plant diseases, not only in those caused by bacteria, but also in those caused by the higher fungi as well. It is known, for example, to be secreted by Peziza sclerotiorum, Aspergillus niger, and Penicillium glaucum when these organisms are parasitic on plants.

Another disease, entirely due to bacteria, is one which affects the tomato, the egg-plant, and the Irish potato. The foliage, and later the stem and leaves, become discoloured, and eventually are destroyed altogether. When the plant is in this condition, the cells are found to be full of bacteria, and if a tiny cut be made in the plant, small drops of a dirty white and yellowish colour ooze slowly out. In potatoes the bacteria make their way to the tubers, causing a brown or black rot. As in most other plant diseases, wounds must first be caused before the bacteria can gain an entrance. In America, where this disease is very prevalent, the wounds are made by an insect called the Colorado potato-beetle, which bites the leaves and, of course, the bacteria first settle on the part of the leaf left exposed by the wound. In recent years this disease has become prevalent in the North of England and in Scotland. The affected tuber is characterised by a pale brown ring inside the tuber at some distance from the outside: the ring later becomes broader and darker. In this way the whole tuber is destroyed, after which the skin withers and many millions of the bacteria contained inside the tuber pass into the soil ready to infect other plants of the same kind. The microbe-parasite is called **Bacillus solanacearum**. The wounds are caused by insects, so the only way of preventing this disease from spreading is by the use of an effective insecticide.

A disease affecting olive trees and called **Olive tuberculosis** is characterised by the appearance of irregular nodulose tubercles on the branches, each about ¼-1 inch in diameter. The tubercles superficially resemble the galls of insect origin, but when microscopically examined, they are found to be full of bacteria. The latter belong to **Bacillus oleae.**

A somewhat common disease is the black rot of cabbage, caused by **Pseudomonas campestris.** The infliction of this disease results in a dwarfing or in a one-sided growth of the heads. Not infrequently it may be characterised by the absence of heads altogether. The stem shows brown or black in the woody portion, whilst the other parts become yellow in colour, and the leaves appear as if their edges had been burnt. The germ enters the margin of the leaves through the water pores. These are small openings found at the ultimate terminations of the veins of the leaves. The plants are weakened by slugs and caterpillars which feed on the leaves, and as they also bear the bacteria in their bodies, they facilitate the dissemination of the disease. The remedy for this disease is to be found in the institution of a rotation of crops, and by checking the multiplication of slugs and caterpillars.

The **Pink Bacteriosis** of wheat is caused by *Micrococcus tritici.* This germ causes the grains to assume a rose or purple colour. The starch is first removed by this microbe with the result that the grain becomes more or less hollow. Next the gluten and finally the cell-walls are attacked. The bacteria are found in an opaque, colourless, thin, and nodulose layer, lining the cavity of the diseased grain.

In the northern and southern parts of France, and also in some parts of England, tomatoes are attacked by a microbe which forms black rings in the fruit. These rings extend in widening circles until the whole fruit is destroyed. The investigation of this disease has not yet been satisfactorily accomplished.

Finally, a disease affecting hyacinths and very prevalent in Holland is caused by a microbe which first attacks the bulbs of these plants when in the resting condition. Later, yellow spots appear on the bulbs and leaves, and a yellow slimy liquid, which teems with bacteria, can be obtained from the inner parts of the plant.

The scientific investigation of plant diseases is still far from complete. The researches that are published on the matter all show that the general conditions affecting the diseases of plants are identical with those that hold in the case of animal diseases. These researches also show that the problem of prevention of plant disease is largely a problem concerning the best means of destroying the creatures that inflict wounds on plants, thus rendering them susceptible to various diseases.

CHAPTER X.

§ 1. HEAT DEVELOPMENT DUE TO BACTERIAL ACTIVITY: THERMOGENIC AND THERMOPHILIC BACTERIA.

As throughout the whole animal and vegetable world, respiration results in a liberation of heat, so we shall expect the same to be true for bacterial respiration: part of the energy derived by the breaking down of food materials, or of the protoplasm, will be expended in the development of heat. The experiments of investigators in this direction have shown the correctness of these deductions. If a culture of bacteria be protected as far as possible from loss of heat by radiation, conduction, etc., it shows a distinct rise of temperature, and exact methods have been devised for estimating the amount of heat liberated. In most cases of bacterial activity such a large amount of energy is needed for purposes of growth, multiplication, etc., which involve an absorption of energy, that much heat is not developed. Again, much of the heat developed during respiration is dissipated by radiation and conduction. Under certain conditions, however, some organisms liberate a very large amount of energy in the form of heat, with the result that a considerable rise in temperature takes place in the medium in which these organisms are growing. In the preparation of beer and wine the rise of temperature during fermentation is well known. Again, a considerable development of heat always follows the massing together of any humid mass of organic matter. We see instances of this in the "smoking" of dung-heaps and in the disastrous effect which follows the massing together of moist hay. In the fermentation of the tobacco leaf the leaves are purposely moistened and then massed together in large bundles in order to secure this rise of temperature. Further instances are exhibited in the preparation, for human consumption, of many of the products of nature, *e.g.* of tea, coffee, cocoa, and many of the spices.

That the rise of temperature in these processes is, partly at least, due to microorganisms may be gathered from the fact that the rise does not occur if the material be sterilised before being massed together. The spontaneous heating of hay and of cotton-waste has been studied more closely than most of the other processes of a similar nature. A rise of temperature occurs in moist cotton-waste only if oxygen be present, so that the development of heat, in the initial stages, has been ascribed to aerobic organisms. The temperature rises to 67° C., and the cotton-waste becomes converted into a humous mass from which vapours of trimethylamine are given off. Very little is known of the specific organisms which help to cause this development of heat.

The spontaneous heating which occasionally takes place during malting, as a result of bad management, is probably due to the activity of a mould called Aspergillus fumigatus. The temperature rises to 60° C. and higher, and there can be no doubt that the diastatic power of the malt is in consequence seriously impaired. The most successful of the researches on this subject has been in connection with the spontaneous heating of moist hops. From the warm mass a microbe has been isolated, and studied in pure cultures. This has been named **Bacillus lupuliperda**. The individuals comprising this species are 0·7 μ in breadth and from 0·7 to 2·5 μ in length: they are motile under normal conditions. The microbe thrives well in hop-extract, excreting large quantities of trimethylamine. In the presence of sugar the cultivating medium is acidified owing to the production of butyric acid. Bac. lupuliperda also liquefies gelatine. Its normal habitat is the soil, from which it enters into the hop, and if during storage the hops are massed together in a damp condition, this microbe multiplies rapidly. The result is spontaneous heating, which greatly deteriorates the value of the hops. Although this microbe has not been isolated from hay, it is very probably an active agent in the spontaneous heating of this substance, as, here also, under the same circumstances, large quantities of trimethylamine are produced. At the same time it must be mentioned that the spontaneous heating of hops is not always, if ever, solely occasioned by the activity of Bac. lupuliperda, as other organisms have been isolated from decomposing hops. Thus a yeast allied to Saccharomyces Ludwigii has been isolated, as well as several moulds, *e.g.* Aspergillus glaucus, Penicillium glaucum, Rhizopus nigricans, and Oidium humuli. Aspergillus glaucus and Penicillium glaucum use up the organic acids of the hops, as well as their sugar, so that they render the reaction of the hops, which is acid in its normal condition, alkaline.

Of still greater economic importance is a knowledge of the conditions which prevent the spontaneous heating of stored corn and other grain. The damping of such substances, carried as cargoes, has caused many a ship to catch fire. The flora on the surface of stored corn has been investigated. Bacteria, yeasts and moulds are found in great numbers; there may be as many as eleven millions per gram. Of course the presence of most of these does not affect the corn one way or the other, but amongst them are some organisms which can take advantage of the corn when in a damp condition and effect multiplication at its expense. A rise in temperature is the result. The amount of moisture present in the corn determines which of the different kinds of organisms obtain the upper hand. If the percentage is between 15 and 25, certain spore-forming bacteria, and if as much as 30 one or more of the moulds, predominate: in either case, a rise in temperature is the result. It is obvious that the best method of protecting stored grain from the ravages of these organisms is to store it in as dry a condition as possible and then protect it from rain and from absorption of moisture from the atmosphere.

The modes of preparation of **burnt hay** and of **tobacco** are described in a later chapter. It is sufficient to note here that the mown grass in the one case, and the tobacco leaves in the other, are massed together in a humid condition in order to promote the desired development of heat.

As is well known, the ultimate result of allowing materials like damp hay or damp cotton-waste to remain massed together is **spontaneous combustion**. The temperature of the humid mass rises to the ignition-point, and bursts into flame. Microorganisms are not, however, able to raise the temperature to the point of ignition, as this is a good deal higher than the thermal death-point of living organisms. Hence other agencies must in addition be invoked to explain the rise of temperature from about 70° C., which is the highest temperature at which bacteria are known to be able to thrive, up to the temperature of ignition. These agencies are very imperfectly understood. It is probable that the most important of these is the activity of the *oxydases* or *oxidising ferments* that are present in the humid vegetable mass. These bring about certain chemical changes which are attended by the liberation of a considerable amount of heat.

We must distinguish between the **thermogenic** or heat-generating and the **thermophilic** or heat-loving bacteria. As already stated, all bacteria generate a certain amount of heat, but those only are called thermogenic which generate so much heat that the temperature of the

medium in which they are growing is raised to a comparatively considerable degree. Bacillus lupuliperda is an example. We know very little of these organisms, though they are present in large numbers in the soil, and it is certain that they have a very wide distribution. It is only when humid organic matter is massed together that they obtain the conditions necessary for active growth and multiplication. It is not improbable that many of the well known soil bacteria must be reckoned among the thermogenic organisms. On the other hand there are bacteria which whilst not developing much heat themselves can not only tolerate, but also actively multiply, at temperatures which stop the growth of other organisms altogether. To these the term "thermophilic" is applied. When the temperature of moist organic matter approaches 70° C. all the organisms in the decomposing mass, with the exception of the thermophilic kinds, have been killed by the heat. Some organisms therefore, for example some of those that are present in such matter when the temperature approaches 70° C., are probably both thermophilic and thermogenic. The best known thermophilic microbe is **Bacillus thermophilus**. This organism thrives and reproduces with great activity at 70° C., a temperature which produces painful burns on the skin, coagulates egg-albumin and kills animal cells. Bacteria have been found in an active condition in boiling springs, for example in one in Mexico the temperature of which is 64° C. We may regard this power of thriving at such high temperatures as having been induced by a long process of acclimatisation, aided by natural selection.

§ 2. CHROMOGENIC BACTERIA.

We may distinguish three kinds of chromogenic or colour-producing bacteria.

1. Those in which the colouring matter is connected in an important way with the nutrition of the cell, such as the purple sulphur-bacteria, and the bacteria which have a green colouring matter. These are called **Chromophorous** bacteria.

2. Those in which the colouring matter is a secretion of the protoplasm which is not extruded from the cell. These are called **Parachromophorous** bacteria.

3. Finally, those in which the colouring matter is an excretion which is thrust out of the cell, the bacteria themselves remaining colourless. These are called **Chromoparous** bacteria.

1. **The Purple Bacteria.** Under this name is comprised a group of organisms differing very much from one another in shape, but all agreeing in possessing a pigment of a purple colour, usually situated just within the cell-wall. This pigment is diffused in the protoplasm and not collected into definite bodies as in the majority of plants showing pigments of this nature. This colouring matter is called *Bacterio purpurin*. The mode of life of these organisms is quite unique in the bacterial world, for they *seek* the light, and do not avoid it, as do the rest of the bacteria. The reason for this will be seen from the following experiment:

A drop of water containing these purple organisms is placed on a slide, and a very small spectrum projected thereon. It is found that the bacteria collect at certain points, viz. in the ultra red, to a smaller extent in the orange, and to a still smaller extent in the green parts of the spectrum. Now the spectrum of bacterio-purpurin shows absorption bands just at these three places, so that these bacteria, like green plants, obtain energy by absorption of light, and it is only certain parts of it, viz. the ultra red, orange, and green components that are absorbed. The bacteria are thus able to make use of the energy of the sun's rays to build up food material. We shall return to these interesting organisms when dealing with the sulphur bacteria to which they belong.

Some chromophorous *green* bacteria have been described, but it is not certain that these are not green algae which have been described as bacteria.

2. **Bacteria producing Red Colouring Matter.** The best and the longest known of these is Bacillus prodigiosus, which in young cultures is parachromophorous, whilst in older cultures it is chromoparous. When, in olden times, red spots were seen on articles of food, it is small wonder, considering the ignorance of those times, that the power of witchcraft was invoked to account for their presence. This bacillus grows on a variety of food stuffs. The colouring matter is at first diffusely distributed throughout the cells, but later is thrust out and collects outside the cell-membranes. This production of colour is dependent on nutritive conditions, for no colour is developed if the organism be cultivated on sterilised potatoes at a temperature of 38°-39° C.

Other well known "red" microbes are, Bacillus erythrosporus, Bacillus Kieliensis, Bacillus lactis erythrogenes, Bacillus corallinus, and Sarcina rosea. When normal milk becomes red on standing, the cause of the malady is usually a development of either Bac. prodigiosus or

Bac. lactis erythrogenes or Sarcina rosea. When other food stuffs, such as cheese, boiled carrots, or boiled meat become covered with red spots, the cause of the malady is, in most cases, traceable to Bac. prodigiosus, each spot being a colony of this microbe which, as just mentioned, shows great adaptability, and can attack a variety of food stuffs.

3. **Bacteria producing Yellow Colouring Matters.** A large number of organisms belonging to the Sarcina group possess the power of secreting yellow colouring matters: among these may be mentioned Sarcina ventriculi, the first to be discovered of that group, Sarcina flava, Sarcina mobilis, Sarcina lutea, and many others. Also they are not wanting in other groups; thus Bacillus synxanthus is sometimes responsible for the production of "yellow" milk—that is, milk which turns yellow on standing. This malady occurs only in milk that has been boiled, so that its growth is probably prevented in unboiled milk by the multiplication of the lactic-acid bacteria which are so abundant in milk, and which are killed when this liquid is boiled. The colouring matter of this microbe is insoluble in alcohol and ether, but soluble in water. It is not affected by alkalies, but acids seem to have the power of combining with it to form a colourless compound. Of the large number of bacteria which secrete yellow colouring-matters, almost all are harmless saprophytes.

4. **Bacteria producing Blue Colouring Matters.** Among the bacteria which produce blue colouring matters one, Bacillus lactis cyanogenus, has been known a long time, and extensively studied on account of its frequent occurrence in milk, to which it imparts a blue coloration when present in excessive numbers. The coloration appears in from twenty-four to seventy-two hours after the milk has been drawn from the cow, the process being hastened in warm weather and retarded in cold weather. The individuals of this species are short, actively motile rods, which have the power of forming endospores. The average breadth of an individual has been given as 0·3-0·5 μ and the average length 1·4 μ. A very small amount of acid is sufficient to kill it, consequently it never attacks sour milk, and when present in sweet milk stops growing when this turns sour. This microbe grows equally well in the milk of the cow, ewe, goat, mare, ass, and dog, and also in human milk. It likewise feeds on many other articles of diet, such as rice, potatoes, etc., and is in consequence very widely distributed. Fortunately it is not pathogenic, and its chief objection is that it gives to articles of food, especially to milk, an unappetising appearance. The colouring matter is excreted outside the cell (chromoparous).

Another microbe of this nature which attacks milk and other food articles is Bac. cyaneo-fluorescens.

A blue coloration, sometimes appearing as patches, at other times permeating the whole mass, is a well-known cheese malady. These defects are sometimes, though not always, due to bacteria, the best known of these being *Bacillus cyaneofuscus*. When cultivated in a nutrient solution a thin pellicle is formed on the surface of the liquid, which, when microscopically examined, is found to be composed of colourless rods. The colour is due to the presence between the rods of blue granules from 1·5 μ to 3·5 μ in diameter. This microbe is therefore chromoparous. As this organism is easily killed by drought, it is not usually found in the atmospheric dust.

A very well known organism belonging to the same class is Bacillus pyocyaneus, which belongs to the pathogenic bacteria. This species usually produces a blue pigment, but when cultivated in certain nutrient media produces a green fluorescent matter, and in other media no colouring matter of any kind.

5. **Bacteria producing Violet and Green Colouring Matters.** Although several chromoparous organisms producing violet colouring matters are known, they have not been extensively studied. Among these the best known are Bacillus violaceus, Bacillus membranaceus amethystinus, and Micrococcus violaceus.

A fairly large number of chromoparous bacteria producing green excretions have also been described. One of these is Bac. fluorescens liquefaciens, which in ordinary bouillon produces a beautiful green fluorescence. If a bouillon culture of this species be placed in the invisible violet end of the spectrum this part is made visible, being seen as a pale yellow colour. Other green fluorescent bacteria are Bac. fluorescens non-liquefaciens, Bac. butyri-fluorescens, Bac. syncyaneus, Bac. viridans, Bac. pyocyaneus.

§ 3. PHOTOGENIC OR PHOSPHORESCENT BACTERIA.

Phosphorescence may be described as the production of light without heat. The term has reference to the fact that phosphorus even under water is luminous, although no perceptible heat is developed such as accompanies the ordinary production of light. This striking phenomenon may be produced in many mechanical ways, *e.g.* by heating, as when certain diamonds are heated to a temperature of 300 -400° C., or by friction, as when two crystals of quartz are rubbed together. In

addition, the phenomenon is exhibited by certain animals and plants, and of the latter a large proportion belong to the bacteria. The first information with regard to bacterial phosphorescence was given us as far back as 1853, when Heller declared that the luminosity of decomposing animal flesh was due to the presence of bacteria. No further advance was made until Pflüger in 1875 carefully investigated the phosphorescent slime that he found on the body of a certain kind of shell-fish. He demonstrated that the slime contained large numbers of bacteria. By filtering a solution of the slime—dissolved in 3 per cent. sea-water solution—and by showing that the filtrate was non-luminous, whereas the filter paper glowed with phosphorescent light, he demonstrated that the phosphorescence was caused by the bacteria that had been stopped by the filter-paper, and not by something else contained in the slime. Since this time our knowledge of the phosphorescent bacteria has been greatly extended; pure cultures have been obtained, and the life-histories of twenty-eight of these organisms have been accurately followed. They are found in all parts of the world and in all climates. They are peculiarly adapted to the conditions that obtain in the sea, for what traveller on the sea is not familiar with the phosphorescence that is observed in the wake of a ship, and the beautiful glow that sometimes lights up the waves as they break on the shore? Eleven of the known twenty-eight species have been obtained out of the sea, and doubtless there are many more that have not yet been described. Again, these organisms are responsible for the phosphorescence that is often observed in rotting fish, in decomposing meat of various kinds and sometimes in rotting wood. It is not uncommon on a dark night to see a beautiful luminous object on the roadside, which on closer examination turns out to be the remains of a decomposing fish. One of the phosphorescent bacteria is parasitic on the blood of sandhoppers, causing a disease which kills them. The stricken sandhoppers shine like glowworms, and by pricking first a diseased creature and then a healthy one it is possible to spread the disease, and consequently the phosphorescence. It is not uncommon for butchers, especially in seaside towns, to notice that their shops are lit up with a faint luminous glow when the lights are turned out; and it has been stated that a large percentage of the fish sold in the markets at Trieste are phosphorescent in warm weather, though apparently no harm accrues from their consumption. Again, cooked potatoes in the first stages of decomposition often glow in the dark. In the case of the meat, the fish, and the potatoes the phosphorescent bacteria are responsible. Finally, cases are recorded, but

are very rare, of persons suffering from tuberculosis, becoming phosphorescent.

The known species belong to quite different families of bacteria. One belongs to the Coccaceae, fourteen to the Bacteriaceae, and fourteen to the Spirillaceae, and of those belonging to the Bacteriaceae some belong to the genus Bacterium, others to the genus Bacillus, and the rest to Pseudomonas.

The commonest and most widely distributed species is Bacterium phosphoreum. As in a large proportion of cases of phosphorescence this organism is responsible for its production, we must single it out for a detailed description. The individuals are rod-shaped with rounded ends, some being so short that they are almost round. The latter kind are about 1-2 μ in length, whilst the longer ones range from 2 μ to 7 μ. Phosphorescence occurs only if free oxygen be present. The optimum temperature of growth is somewhat low, being about 16°-18° C. This microbe does not grow above 28° C., hence the necessity of cultivating it in a cold room. Like other bacteria it can stand very low temperatures without injury. The light which it emits is of a bluish-green colour, and can be intensified by the addition of small quantities of common salt, potassium nitrate, or potassium chloride. On gelatine plates whitish-yellow phosphorescent colonies with slightly wavy edges are formed. A gelatine stab shows the same type of growth on the surface, and as is to be expected from an aerobic organism, very little development down the stab. Cultivations can easily be made in nutrient agar, on potato, and in milk, in all of which a yellowish-white phosphorescent growth results. Endospore formation is unknown.

Phosphorescence can be easily obtained by half covering a chunk of beef with a 3 per cent. solution of common salt, and leaving it in a cold, damp room. After one to three days the meat will probably be seen to glow with a bright phosphorescent light. This light is seldom found extending over the whole surface of the meat, but rather emanates from a number of distinct spots. There will be as many of these spots as phosphorescent bacteria that have fallen on to the meat, for each spot of light represents a colony of these bacteria, and each colony has been derived by the growth and multiplication of one individual phosphorescent microbe.

It is very probable, if this experiment be made, that the organism causing the phosphorescence will be found to be Bacterium phosphoreum, for this species is most abundant in market places, in slaughter-houses, and in kitchens and cellars. A cultivation of this

species may be effected by making use of a mixture prepared in the following way: To a litre of water 125 grams of minced beef are added. After being shaken, the mixture is allowed to stand at about 10° C. for a day or so, when the sap is pressed out and about 3 per cent. of common salt added. The mixture is next boiled and filtered, and to the filtrate 10 grams of peptone and 100 grams of gelatine are added. Then caustic soda is added, drop by drop, until a very faint alkaline reaction is obtained. The mixture is completed by the addition of 0·5 per cent. of glycerine. By inoculating this mixture with a little of the phosphorescent flesh, and then making gelatine-plate cultures from the resulting growth, pure cultures may be obtained.

Physiological Considerations. It will be noticed that common salt is an important ingredient of any mixture for the cultivation of phosphorescent bacteria. They will not grow in the ordinary nutrient media, but the addition of even 0·5 per cent. of common salt immediately changes the aspect of affairs, and growth and multiplication actively set in. This partly explains why the sea is such a favourable habitat for their multiplication. Experiments have shown that about 3 per cent. of common salt produces the optimum effect. If more than 3 per cent. be used the resulting growth is smaller, whilst if 10 per cent. or more be employed no growth at all takes place. It has been recently found that the salt can be replaced by a number of other substances. Common salt is the chloride of sodium, and it has been shown that any chloride, *e.g.* of potassium or of magnesium or of calcium, can be substituted for it. Further, even a chloride is not necessary, for instead, potassium nitrate or potassium iodide will serve the purpose.

There is no necessary connection between phosphorescence and either growth or respiration. Thus, in the case of Bacterium phosphoreum, the addition of small quantities of magnesium sulphate greatly increases the growth, but the phosphorescence remains very weak. On the other hand, a minute addition of laevulose or glucose causes an increase in the phosphorescent glow after a few seconds, and in this short time there could be no perceptible change either in the growth or in the respiration. Many substances, however, *e.g.* common salt, favourably influence together the growth, the respiration and the phosphorescence.

Beijerinck has proposed to utilise these bacteria as aids in the identification of certain carbohydrates and of certain enzymes. His proposition was to make use of the different behaviour of Photo-

bacterium[1] phosphorescens and Photobacterium Pflügeri towards maltose, as a means of ascertaining whether this sugar was or was not produced in any diastatic process. For this purpose a mixture composed of 100 c.c. sea-water, 8 per cent. gelatine, 1 per cent. peptone, and 0·25 per cent. potato-starch is prepared. Then plate-cultures of these organisms are prepared, using this mixture as the nutrient medium. Suppose we wished to test whether a certain solution had diastatic properties, a few drops would be placed on each plate. If these drops contained diastase this would act on the potato-starch and produce maltose. The production of this substance would have no effect on the culture of Ph. Pflügeri, because this organism is indifferent to maltose, but on the culture of Ph. phosphorescens the presence of maltose will at once be signalised by a perceptible development of phosphorescence. The method has also been used to ascertain whether any other microorganism has the power of forming enzymes, and also for finding out the nature of these enzymes. For this purpose several gelatine plates of Ph. phosphorescens are prepared. Some of these receive each a couple of drops of the sugar lactose, others the same quantity of cane sugar, and a third lot raffinose. Not one of these sugars can be assimilated by Ph. phosphorescens. The drops are absorbed into the gelatine, and form patches, called by Beijerinck *diffusion-fields*. Near each diffusion-field the gelatine is inoculated with the organism that is to be tested. This will grow into the diffusion-field. When this experiment is tried with Saccharomyces Kefyr it results in all three sets of plates becoming phosphorescent. Now, as phosphorescence appears only after the introduction of Sacchar. Kefyr, it shows that this organism is able to change the three kinds of sugars into other forms which can be absorbed by Ph. phosphorescens and produce phosphorescence; that is to say, enzymes are secreted by Sacchar. Kefyr, which bring about the change from one sugar to another and more assimilable form.

Cause of Phosphorescence. There is still a good deal of dispute as to the cause of the luminosity of bacteria. According to one theory, the body of the microbe is dark, the luminosity being produced by a substance excreted by it. This theory is supported by the fact that certain chemical substances, *e.g.* methyl aldehyde and grape-

[1] Beijerinck uses the generic term "Photobacterium" to cover all the phosphorescent bacteria, irrespective of their shape or any other characteristic. The use of the term is not advisable for purposes of classification, and even for descriptive purposes is apt to mislead, owing to the introduction of the word "bacterium."

sugar become phosphorescent in slightly alkaline solutions. In these cases the phenomenon of phosphorescence goes hand in hand with the process of oxidation that takes place. It is assumed by this theory, that the same process takes place in phosphorescent bacteria. The theory is further supported by the facts that these bacteria are luminous only in alkaline media, that phosphorescence is observed in the presence of compounds that are either aldehydes or nearly related to the aldehydes (*e.g.* the sugars) and finally that luminosity is observed only in the presence of oxygen. Marine phosphorescence, however, is more difficult to explain, for there cannot be sufficient aldehydes or related bodies in the sea to produce the phosphorescent effects that are there observed.

The other theory is that the phosphorescence is a form of energy, and does not emanate from any particular substance. It is supposed to be possessed by these bacteria, just as other plants possess the power of movement, that is to say, phosphorescence is a vital phenomenon, bound up with the living protoplasm and incapable of separation from the same. This theory has fewer facts to support it than the other, but it affords a better explanation of marine phosphorescence.

The colour of the phosphorescent light is white or yellowish-white and green or bluish-green. Further, the colour depends on the nature of the nutrient medium and the position of the eye. Unlike the spectrum of the sun, that of phosphorescent light is continuous, that is to say, it is not interrupted by dark absorption lines and bands. The spectrum extends in some species from *b* (green) up to violet, and in other species from *D* to *G*, the blue and violet rays being most prominent. The light differs from that of phosphorescent animals in that it is steady, and a good culture may exhibit a glow for months after inoculation. The phosphorescence from most animal organisms, on the contrary, lasts only for a short time, a few seconds or a few minutes, and in some cases is exhibited as a result of an external stimulus. The light emitted by phosphorescent bacteria suffices not only to photograph themselves, but also surrounding objects. A darkening of the photographic plate can be effected by laying it on the surface of a photogenic culture for as short a time as one second. A more interesting practical application is the construction of **bacteria-lamps**, and in the Paris Exhibition of 1900, a whole room owed its light to the presence of a number of such lamps. Their use as night-lights has been suggested, but an apparatus of this description is more of a scientific curiosity than anything else, and has not become a commercial success.

Finally, we may mention that plants which in their growth bend towards the light, if this strikes them laterally, *e.g.* plants placed to grow near the window, will likewise bend towards a phosphorescent light. If some peas be placed in a pot, and the latter be placed near a gelatine plate-culture of phosphorescent bacteria, the young stems, as they emerge from the soil, will bend towards the phosphorescent light.

CHAPTER XI.

THE SULPHUR-BACTERIA.

§ 1. INTRODUCTION TO SULPHUR-BACTERIA.

The Sulphur-bacteria perform excellent service in nature, for, by their aid, substances absolutely useless in themselves for plant life are changed into substances which can again be used up by plants. As has been explained, a large number of bacteria have the power of breaking up albuminous materials, with the result that the sulphur bound up in their molecules escapes as sulphuretted hydrogen (H_2S). The decomposition of eggs is a good example of this action. The emanation of sulphuretted hydrogen can easily be tested by holding a piece of lead paper above some decomposing liquid, when the paper will turn black if this gas be given off. This property of decomposing albuminous substances, with evolution of H_2S, is common to almost all bacteria which feed on these substances. This gas may also arise in a variety of other ways, thus sulphates may be reduced to the sulphide by such bacteria as Vibrio hydrosulfureus, Proteus vulgaris and others: again, thiosulphates may be changed, with formation of sulphuretted hydrogen, by Vibrio hydrosulfureus and other bacteria, and, according to Beijerinck, sulphites also may be reduced in the same way under similar conditions. As is to be expected, therefore, a large quantity of sulphuretted hydrogen is being produced daily, as the result of this decomposition, and the places in which the sulphur-bacteria are to be found are just those waters in which sulphuretted hydrogen is present. They are therefore principally found in sulphur springs. They form a delicate matting to the bed of the spring, the matting being most commonly snow-white, but sometimes red or reddish-violet. They are best studied late in autumn or at the beginning of the year, when the

vegetation of the previous summer has had time to reach a high stage of decomposition. At these times they may be seen on almost every decomposing ditch or pool. The presence of decomposing vegetable and animal remains in still waters explains why they are often found in sea-water pools on the coasts. Winogradsky gives the following method for cultivating these bacteria: Small portions of the water-plant, Butomus umbellatus, together with some mud from the place from which the plant was collected, are placed in a deep vessel, and to these is added some 3-5 litres of water. Next about 2 grams of gypsum ($CaSO_4$) are added, and the whole, uncovered, is put aside at the temperature of the room. After 5-7 days, the development of sulphuretted hydrogen will be noticed, as a result of the reduction of the gypsum by some bacteria present in the water. These prepare the medium for the sulphur-bacteria. After 3-5 weeks the medium will be found to contain an abundance of sulphur-bacteria.

§ 2. CLASSIFICATION OF THE SULPHUR-BACTERIA.

All bacteria, inside the cells of which sulphur may be found, are grouped together under the term **Sulphur-bacteria**. The group is not a natural one, for the organisms composing it have no characteristics in common, other than the power of assimilating sulphuretted hydrogen, and effecting its oxidation in the manner mentioned above. This grouping is, however, very convenient in many ways.

The bacteria are divided into the following genera:

1. **Beggiatoa.** Colourless, motile thread-bacteria.
2. **Thiothrix.** Colourless, motionless thread-bacteria.
3. **Thiophysa.** Colourless bacteria with no formation of threads.
4. **Purpur-bacteria.** Coloured bacteria.

I. **Beggiatoa.** The sulphur threads classed under Beggiatoa are cylindrical, and actively motile. They may attain even a centimetre in length. Inside the threads, at a certain stage, are seen round, strongly refractive sulphur bodies. No transverse walls can be seen at this stage (Fig. 82*a*), but they are developed later after the sulphur has disappeared from the threads (Fig. 82*c*). When the supply of sulphuretted hydrogen runs short the threads break up, as represented in Fig. 82*d*, which is the first sign of their approaching death. Spore formation is as yet unknown.

The genus includes the following species:

Beggiatoa Alba. Threads, 2·8–2·9 μ thick. Length of individual cells, 2·9–5·8 μ.

Beggiatoa Media. Threads, 1·6–1·7 μ thick. Length of individual cells, 4–8·5 μ.

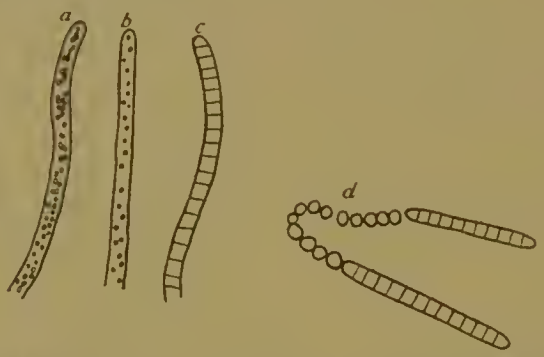


Fig. 82.—Beggiatoa alba. (*a*) In medium containing a plentiful supply of sulphuretted hydrogen. (*b*) In medium devoid of sulphuretted hydrogen: appearance after twenty-four hours; sulphur granules rapidly disappearing. (*c*) Appearance three days after deprivation of sulphuretted hydrogen; sulphur has completely disappeared and transverse walls have been formed. (*d*) Filament breaking up into fragments. (After Winogradsky.)

Beggiatoa Minimum. Threads, 0·8 μ thick.

Beggiatoa Mirabilis (Fig. 83). Cylindrical, very actively motile threads. As the thickness of the thread measures up to 45 μ, the individual cells can be seen with the aid of a small magnification. The length of the cells is about half the thickness of the thread.

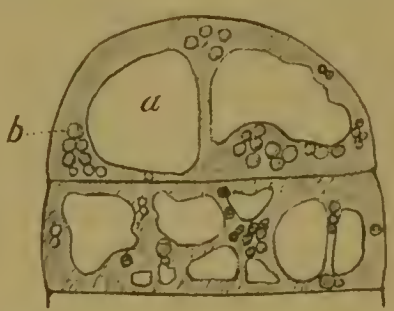


Fig. 83.—Beggiatoa mirabilis. (*a*) Vacuoles. (*b*) Sulphur globules. (After Hinze.)

This gigantic organism has a sharply contoured wall, and the cell contents show a number of vacuoles in the protoplasm, just as we see in mature cells of higher plants. These vacuoles are filled with cell-sap.

II. **Thiothrix.** This genus is distinguished from the preceding by its immobility. One end is always attached by a mucilaginous cushion secreted by itself, to any convenient object, a stone for example, the

other end lying free in the water. The division of the thread into cells is usually hidden by the large quantity of sulphur, but the cells can be demonstrated by washing out the sulphur with absolute alcohol, and then staining with fuchsin or methylene-blue. The cells towards the free end are somewhat larger than those at the other end. Another mark which distinguishes this genus from Beggiatoa is the presence of a common thread-membrane in addition to the membrane which each cell possesses. Thus it follows that Beggiatoa in breaking up divides into separate parts, whereas the Thiothrix cells are more or less held together inside the thread membrane. Still another distinguishing mark is that at the free end, single cells loosen themselves and, after becoming separated from the parent thread, slightly elongate themselves, and then develop a mucilaginous, adhesive cushion. As this is done by a large number, and adhesion takes place near the parent thread, a thick cluster of threads of characteristic appearance soon results. Three species have been described.

Fig. 84.—Thiothrix nivea. Group of young threads containing sulphur granules. (*b*) Mucilaginous cushion.

1. Thiothrix Nivea. Threads 2–2·5 μ thick at the base, 1·7 μ thick in the middle, and 1·4–1·5 μ at the apex (Fig. 84).
2. Thiothrix Tenuis. Threads equally thick, usually 1·0–1·1 μ.
3. Thiothrix Tenuissima. Very small threads, average of 0·4–0·5 μ thick.

As these organisms are motionless, and as no sulphur bacteria can grow without a supply of free oxygen, they are usually found at the bottom of fast-flowing streams, because, if the sulphur-containing water is still, the supply of oxygen in it is scanty and chiefly at the top. Hence it is accessible only to the motile genus Beggiatoa. On the other hand, if the water flows at a rapid pace, the supply of oxygen at the bottom is greater and therefore permits of the growth of representatives of this genus.

III. **Thiophysa.** In this group are placed all the colourless sulphur-bacteria which do not form threads. So far back as 1876 Warming described two forms, known respectively as Monas Müllеri and Monas fallax, the former spherical, with a diameter of 5·6–15 μ, the latter ellipsoidal, 4·5 μ long and about 3 μ broad. Two others were discovered later by Jegunow, both having a slightly bent, rod-shaped

structure. The shape of another species, described by Hinze, is seen in Fig. 85. This, on account of its motility, was named Thiophysa volutans. The diameter of this form varies from 7 μ to 18 μ. This group has not been extensively studied; it is doubtful whether the

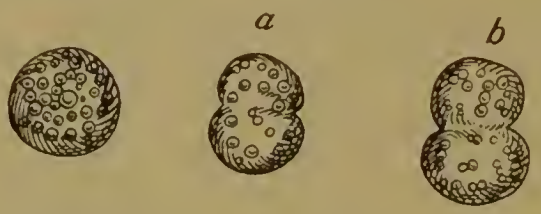


Fig. 85.—Thiophysa volutans. (*a*) and (*b*) In process of division. (After Hinze.)

various species included in this group are really members of the same family, in the sense of being phylogenetically connected.

IV. **Purpur-bacteria.** The purpur-bacteria are characterised by having a colouring matter inside the cells. The name *bacteriopurpurin* has been given to this colouring matter by Ray Lankester. The chief forms of purpur-bacteria are the following:

1. **Chromatium okenii** (Fig. 86*a*). A cylindrical cell 10-15 μ long and 5 μ broad. The motility of this form is due to the possession of one cilium.
2. **Monas Warmingii** (Fig. 86*b*). This is not unlike Chromatium okenii in appearance, differing slightly in size and shape.

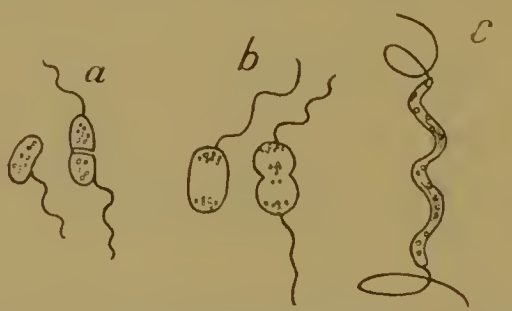


Fig. 86.—(*a*) Chromatium okenii; (*b*) Monas Warmingii; (*c*) Spirillum volutans. (After Cohn.)

3. **Spirillum volutans** (Fig. 86*c*). There is a good deal of confusion with regard to this species. The organism commonly called by this name is in reality Spirillum giganteum, which does not contain sulphur, though a large quantity of fat and volutin is commonly present. The species originally called Spirillum volutans has never been isolated and has not recently been observed. It is, therefore, doubtful whether it should be included in this group.

4. **Ophidomonas sanguinea** (Fig. 87*a*). Also a doubtful form, but described by Ehrenberg as one of the sulphur-bacteria.
5. **Rhabdomonas rosea** (Fig. 87*b*). Roughly spindle-shaped, as seen in the diagram. About 4-5 μ broad and 20-30 μ long.

This is an interesting group on account of the colouring matter, bacteriopurpurin. It has been stated that it plays the same rôle as the chlorophyll of green plants, by the aid of which the latter are able to elaborate carbohydrates from the carbon dioxide and water vapour of the atmosphere, the process being also marked by the liberation of free oxygen. Though it has not been conclusively demonstrated that bacteriopurpurin possesses this attribute, it is significant that these bacteria, when cultivated in a glass vessel, congregate in large numbers on the side nearest the light, in this respect differing from all other sulphur-bacteria. Also, when grown in deep vessels, it is noteworthy that the most luxuriant growth is found at the bottom, where the supply of oxygen is very scanty. Now, all living creatures—with a few exceptions—require oxygen for purposes of respiration, so that it seems probable that these bacteria are enabled to live at low depths because they use up the oxygen given off in the process of assimilation mentioned above.

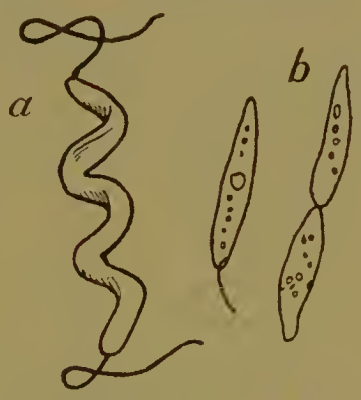


FIG. 87.—(*a*) Ophidomonas sanguinea; (*b*) Rhabdomonas rosea. (After Cohn.)

§ 3. THE PHYSIOLOGY OF THE SULPHUR-BACTERIA.

As has been explained above, these bacteria grow in waters rich in sulphuretted hydrogen. The first change is the conversion of this substance into free sulphur, this taking place according to the following equation:

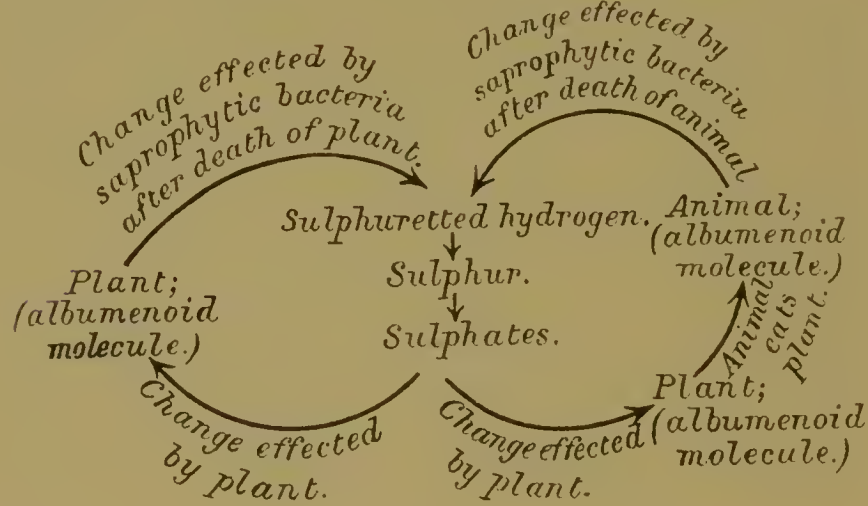
$$\underset{\text{sulphuretted hydrogen}}{2H_2S} + O_2 = 2H_2O + \underset{\text{sulphur}}{S_2}.$$

The sulphur is now stored in the cells and, when required, it is further oxidised into sulphuric acid, according to the equation:

$$\underset{\text{sulphur}}{S_2} + 3O_2 + 2H_2O = \underset{\text{sulphuric acid}}{2H_2SO_4}.$$

It will be noticed that a double process of oxidation takes place. According to Winogradsky, the transformation of sulphuretted hydrogen into sulphuric acid is the process which the plant relies upon

to obtain the energy it needs for carrying on all the processes of life, so that the sulphur-bacteria obtain the same benefit from this process that other bacteria obtain by respiration. It is obviously not identical with respiration, for it does not involve the breaking down of protoplasm. As the end product is sulphuric acid, growth would soon be inhibited altogether if this substance were allowed to accumulate. The acid, however, is neutralised by the acid carbonate of lime, $CaH_2(CO_3)_2$, which is usually present in the kind of water in which these organisms grow. A very interesting result follows from the fact that the two gases which are necessary for the existence of these bacteria, viz. sulphuretted hydrogen and oxygen. are found, the former at the bottom where decomposition is taking place, the latter



at the top where the liquid is in contact with the atmosphere. As both are necessary the organisms are found at a level in the liquid where oxygen has penetrated downwards and sulphuretted hydrogen upwards. If the latter gas is profusely developed the organisms can even be found at the surface, while on the other hand if the supply be very small, they are found at the bottom of the vessel in which they are growing. Some beautiful experiments have been performed by Winogradsky and Jegunow. By varying the supply of these gases, they have been able to control the growth-level taken up by the organisms. We may note here the life-history of the sulphur molecule. As is well known, matter cannot be destroyed. The sulphur is bound in a complicated fashion in the albuminoid molecule in the animal or vegetable body. When the organism dies, complicated changes take place through the agencies of bacteria, resulting in the liberation

of sulphuretted hydrogen. This is taken up by the sulphur-bacteria, and ultimately changed into sulphates. The sulphates are then taken up by the roots of plants and built up once more into the albuminoid molecule. This can be diagrammatically represented as shown above. This, of course, does not represent the whole of the changes that take place, only the broad cycle of events.

THE IRON-BACTERIA.

§ 4. INTRODUCTION TO IRON-BACTERIA.

The iron-bacteria are so called because the membrane of these organisms is usually covered with a reddish-brown deposit of ferric hydroxide. They abound in iron waters, and hitherto have not been found growing elsewhere. Such waters usually ooze out of the earth, the iron in them being present at first in the form of the soluble bicarbonate, $FeH_2(CO_3)_2$: they are common in almost all parts of Great Britain, and particularly in the neighbourhood of Glasgow. The immediate neighbourhood of the spot where this kind of water wells out is covered with a reddish-brown deposit. In the same way the beds of the streams which are fed by this water become reddish-brown in colour. The colour is due to the formation of the insoluble ferric hydroxide into which the soluble bicarbonate changes as it reaches the surface. This change can be represented as follows:

$$\left.\begin{matrix} 2FeCO_3 \\ H_2CO_3 \end{matrix}\right\} + 3H_2O + O = \underset{\text{ferric hydroxide}}{Fe_2(OH)_6} + 2CO_2.$$
$$H_2O + CO_2.$$

The accumulation of the precipitated ferric hydroxide is often a source of trouble, and it has either to be periodically removed, or else conducted into a stream, so that it may be carried down with the water.

If, now, the reddish-brown deposit be microscopically examined, the ferric hydroxide will be found, in the vast majority of samples, to be deposited on the membranes of organisms; in fact, it may be stated that the deposit *consists* of organisms, the membranes of which are coated with ferric hydroxide. Sometimes this coating is so thick that its width is four or five times that of the organism which it envelops. In almost all cases the organisms belong to the bacteria. In some

samples of iron-water however, the deposit is laid on diatoms or allied organisms, though such samples are not common. In a very small percentage of iron-water samples there is no trace of organic life.

Crenothrix polyspora (Cohn). The best known of the iron-bacteria is Crenothrix polyspora, which was first described by Cohn in 1870, attention being then directed to it by the fact that the drinking water in the neighbourhood of Breslau had suddenly assumed a deep red colour. In 1878 the Berlin drinking water was similarly affected, the course of the water being for a time completely choked up by the deposition. Since this time, the same phenomenon has been witnessed in various parts of Germany, France, Russia, and Great Britain. In this country the best known visitation of this nature is that which befell Cheltenham in 1896, when the water in the reservoirs of this town suddenly assumed a deep red appearance. The water was red for about three months, with apparently no danger to the public health. In all these cases the redness was ascertained to be caused by a deposition of ferric hydroxide on the membranes of myriads of individuals belonging to this organism.

Crenothrix polyspora is thread-like in structure, each thread being made up of a single row of cells (Fig. 88), and invested by a delicate sheath. The sheath is formed by the splitting up of the cell-membrane into two layers, of which one becomes the membrane of the cell itself, whilst the other contributes to the sheath-membrane. The latter is therefore the result of contributions from each of the cells enclosed by it. As seen in Fig. 88, the threads are not perfectly cylindrical, as the lower part has a narrower diameter than the upper portion. Also the cells of the lower part are longer but narrower than those higher up the thread. Attachment is by the narrow end. The diameter of the thread varies from 1·5 μ to 5 μ at the base, and from 4 μ to 9 μ at the top. In very young threads, the sheath-membrane is very delicate, and in the youngest of all, absent altogether. With regard to the method of reproduction, according to Zopf, the upper part of a thread divides up to form a comparatively large number of cocci. These vary in size according to the rapidity of division: the smaller are called *micrococci*, and the larger *macrococci* (Fig 88). They vary from 1 μ to 6 μ in diameter. When set free from the thread, the walls of the cocci often swell, with the result that many of them become fastened together, forming a *Zoogloea* (Fig. 88*f*). The thread is gradually emptied of its cocci, after which it collapses and disappears. If the conditions are favourable, the cocci do not form a zoogloea, but instead each

elongates to form a new thread. Sometimes the cocci elongate whilst still attached to the parent thread, with the result that the latter assumes the appearance presented in Fig. 89. According to

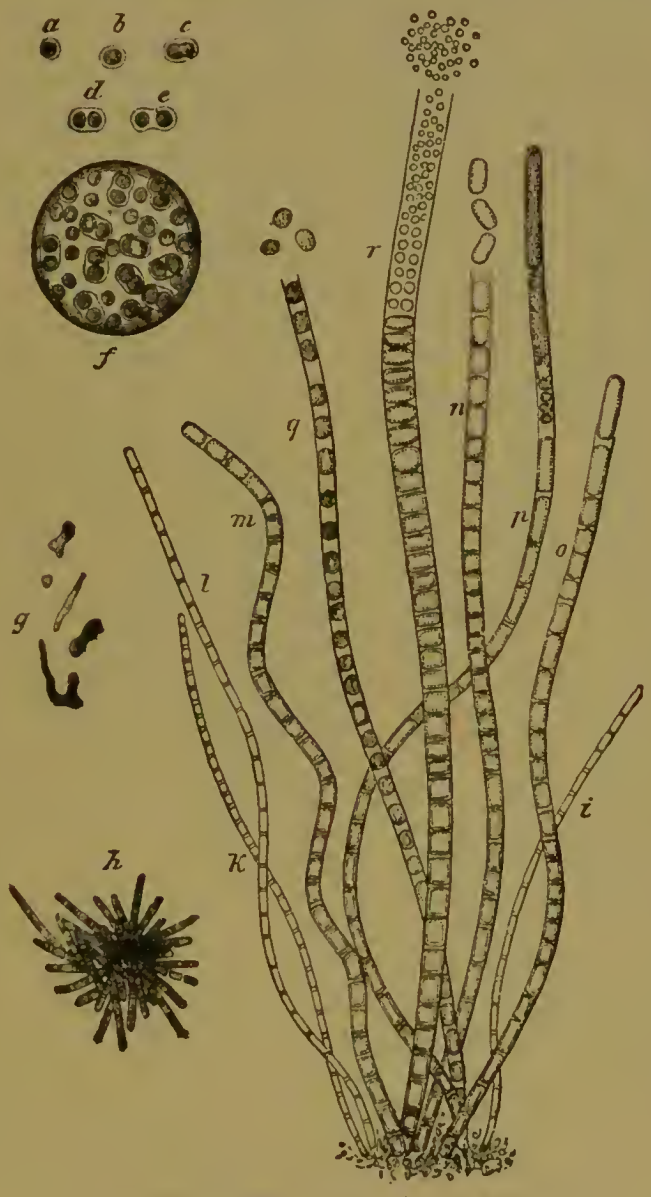


Fig. 88.—Crenothrix polyspora. (After Zopf.)

Garrett, who investigated the Cheltenham water, when this was reddened by the multiplication of Crenothrix polyspora, the cocci are capable of reproducing themselves in the same way as do the cocci belonging to the Coccaceae, each dividing up to form daughter-cocci. He considers that the species is maintained in this way, until

conditions arise which enable them to form filaments and zoogloeae. He also maintains that a mature filament is often produced, not by the division of a single thread, elongated from a coccus into small cells, but rather by the arrangement of cells formed from different cocci into a row. The whole life-history of this organism is not yet thoroughly elucidated. It is widely distributed, probably in the

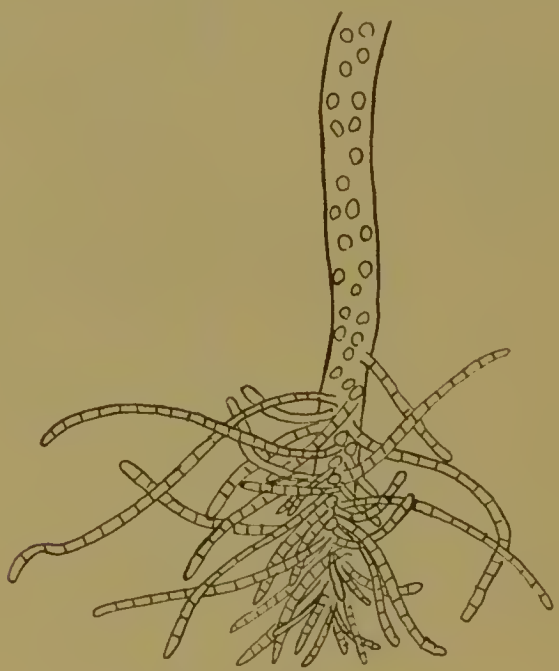


Fig. 89.—Crenothrix polyspora.

cocens form, but its identification, except when, as at Cheltenham in 1896, it multiplies to an inordinate extent, is extremely difficult, if not impossible.

Another well known organism belonging to this group is **Cladothrix dichotoma** (Cohn), which was discovered by Cohn in 1875. It has the same habitat as Crenothrix polyspora. In 1888, in conjunction with this species, it caused much trouble in the Rotterdam Waterworks. Its general appearance is represented in Fig. 90. The threads of the organism form a tree-like tuft of threads. In Fig. 91 the threads are represented on a larger scale. As is the case with Crenothrix, each thread is made up of a number of cylindrical cells, all held together by a common membrane. The branching is of a peculiar nature, each branch being formed by the slipping aside of one cell in a thread, which then elongates and divides to form a new thread, whilst still partly attached to the parent thread. We

cannot call it a real branch, because what has been formed is in reality a new thread which has not yet become completely detached from the parent thread. This peculiar tree-like structure easily

Fig. 90.—Cladothrix dichotoma. (After Zopf.)

Fig. 91.—Cladothrix dichotoma. Portion of thread highly magnified. (After Zopf.)

distinguishes this form from all other bacteria. The average thickness of a thread is about 2 μ. The sheathing membrane is delicate but clearly visible. Multiplication takes place by the formation of what are known as *rod-gonidia*. These are the cells inside the sheath which have become rejuvenated: each develops a bundle of cilia (Fig. 92) and, leaving the filament by their aid, swims about for some time, but ultimately settles fast to some object, and then elongates and divides to form a new thread. An interesting feature in connection with this organism is that a pure culture has been obtained by growing it in gelatine and flesh extract. Circular white specks appear in the gelatine-plates, which later become irregular and send out arms in all directions.

Fig. 92.—Cladothrix dichotoma.

Leptothrix ochracea (Kützing) *syn* **Chlamydothrix ochracea** (Migula). This is the commonest of the iron-bacteria, and is found in every country in Europe. In nearly all the samples of iron-water that have been examined, this species is the predominating organism. It was discovered as early as 1843 by Kützing, who placed it among the algae, but a later investigation resulted in a transference to the thread-bacteria (Chlamydobacteriaceae). The organism is cylindrical in shape, with a fairly thick membrane, which is usually sharply outlined both on the inside and on the outside (Fig. 93). Although each individual is not attached to any object, it is not unusual in a young growth of this organism to find a large colony attached as a whole to the bed of a small brook. In such a colony the individual members are connected together to form an irregular network, the whole swaying to and fro in the water. Even in a network of this nature the ends of the individual organisms are free. Fig. 94 represents the mode of attachment of the individual organisms with one another. This appearance is seen only in comparatively young growths in which the deposition of iron has not as yet taken place to a very large extent. After a while the continuous deposition of iron on the surface of the organisms makes the weight so heavy that the whole colony collapses, and henceforth each thread lies prostrate on the bed of the stream.

Fig. 93.—Leptothrix ochracea.

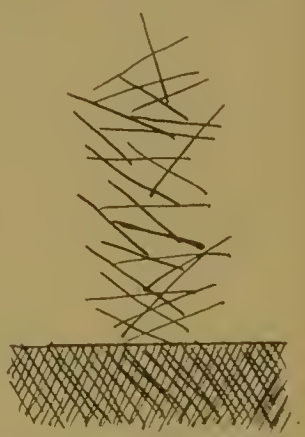


Fig. 94.—Leptothrix ochracea. Diagrammatic representation of colony of young threads attached to bed of stream.

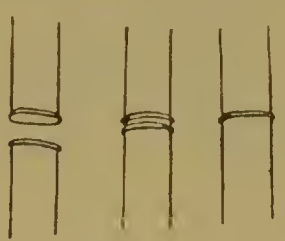


Fig. 95.—To illustrate method of division in Leptothrix ochracea.

The length of a Leptothrix thread varies considerably: the average is from 100 μ to 120 μ. The average width is 2–2·5 μ, though owing to the deposition of iron on the membrane it often appears to be much more. The thickness of the wall also varies considerably, being thin and delicate in young individuals, and thicker and more sharply defined in the older threads.

Multiplication is effected either by a process of division or by the

formation of the reproductive bodies called *conidia*. When a thread is about to divide, two circular thickenings are formed, very close to each other (Fig. 95), and it is between these that division takes place. As the thickenings are often not transverse, but somewhat oblique, threads like the one represented in Fig. 96 are not uncommon. Each of the two portions of the thread elongates after separation, and subsequently divides in the same way. A conidium is formed in the following way: A protuberance appears on the outer part of the membrane; this elongates to a certain length, and is then cut off by a process of constriction. A single thread may form hundreds of conidia, and as most of them remain attached to the thread, the latter often becomes invisible, being surrounded by a dense mass of these bodies (Fig. 97). Various stages in the development of a conidium are given in Fig. 98. The germination of a conidium very probably takes place by direct elongation. Sometimes germination takes

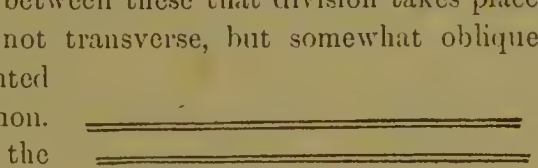


Fig. 96.—Leptothrix ochracea. Showing oblique ends. (See text.)

Fig. 97.—Leptothrix ochracea, covered with conidia.

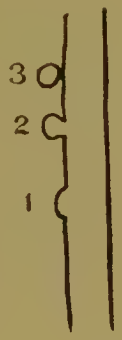


Fig. 98.—Leptothrix ochracea, stages in development of conidium.

Fig. 99.—Leptothrix ochracea. Showing germination of conidia *in situ*.

place whilst the conidia are still attached to the parent thread, with the result that the latter appears to be beset with a number of quill-like structures (Fig. 99).

Gallionella ferruginea (Ehrenberg), *syn.* **Chlamydothrix ferruginea** (Migula). Though this species has been known since 1836 it is only

lately that it has been accurately studied. Each individual consists of a long fine thread, which winds in such a manner that it looks like a hairpin closely and spirally twisted round itself. All sizes can be seen in a favourable culture, from very small ones (Fig. 100*b*) to much larger examples (Fig. 100*a*). It usually occurs in very small numbers along with Leptothrix ochracea, the latter usually greatly preponderating; very rarely it forms the chief organism in iron-water. The thread is not divided in any way, and the ends are rounded off like those of bacilli. According to Migula a very delicate external membrane is present, though later observers have failed to find it. The average diameter of a thread is about 1 μ, but may be as small as $\frac{1}{4}$ μ and as large as 1·5 μ. The loops formed by the twisting vary considerably both as to size and number. There may exceptionally be as many as 35 belonging to one thread.

Fig. 100.—Gallionella ferruginea.

The method of multiplication is of two kinds. The commoner of the two is by division. Small portions are cut off, which immediately curl up, and by growth produce the normal thread (Fig. 100*b*). The other method is by the formation of *conidia*, which arise in exactly the same manner as in Leptothrix ochracea. Examples of conidia formation are shown in Fig. 101. It is interesting to note that preparatory to conidia formation the coils somewhat loosen themselves, so as to allow a greater surface to come into play for this purpose. The conidia are slightly oval, about 1 μ in breadth and 1·5–1·75 μ in length. The stages of germination have not yet been observed.


Fig. 101.—Gallionella ferruginea. Showing conidium-formation.

Clonothrix fusca (Schorler). In 1904, another member of this group was found in the water works in and about Dresden. It combines the characters of Crenothrix and Cladothrix in that while the individual threads are like Crenothrix polyspora in shape and in their methods of reproduction, they are like Cladothrix dichotoma in forming false branches. The threads are unequally thick, the bottom part measuring 5-7 μ in thickness, whilst the upper part may be anything up to 2 μ. The cells in the thread are normally 6-8 μ long, but

sometimes as much as 12-16 μ and even 20 μ. Multiplication takes place as in Crenothrix polyspora by the splitting up of the cells into a number of small motionless bodies, each of which is capable of developing into a new organism.

Spirophyllum ferrugineum (Ellis). This member of the group was first observed near Glasgow, but though very seldom present in large quantities, is widely distributed in Great Britain, being found as far south as Kent and as far north as the Orkneys. Each individual takes the form of an elongated, flattened and spirally-twisted cell, a typical example being represented by Fig. 102. There may be only a quarter of a turn, or there may be fifteen or more. The width various, from 1 μ to 6 μ, whilst the length may reach anything up to 200 μ and possibly more. There is no definite cell-membrane, protection being afforded by the great thickness of the cells at the edges. This is shown diagrammatically in Fig. 103*a*. This is probably a great help in protecting the cell from tearing when it twists itself spirally, especially in view of the fact that the twisting continues after a fairly large amount of iron has been deposited on the cell. The ends of the cells are usually angular and irregular. The spirals may be close or wide apart.

Fig. 102.—Spirophyllum ferrugineum.

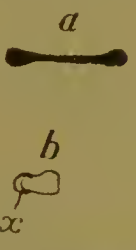


Fig. 103.—Spirophyllum ferrugineum. (*a*) Cross-section, showing thickened margin; (*b*) germination of conidium; *x* = conidium-coat.

Conidia formation, which takes place in precisely the same manner as in Leptothrix ochracea, is the usual method of multiplication. Large numbers are formed, and when, as often happens, these remain attached to the parent plant, the latter is completely hidden, so great is the mass of these bodies. Each conidium has a single coat, and measures 1 μ in width and 1·75 μ in length. In germination the coat

Fig. 104.—Spirophyllum ferrugineum. Two intertwining individuals.

bursts, allowing the contents to protrude (Fig. 103*b*). Very soon after germination the young cell assumes a flat irregular shape, begins to twist, and also to exhibit an independent movement, partly of a wriggling and partly of a pendulum nature. At a slightly later

stage in the development the thickened edge is developed, and movement stops. It is probable that multiplication by cell-division also occurs, but so far the process has not been observed.

Not only do the individuals twist round themselves, but also round other individuals of the same kind (Fig. 104). The twisting is probably due to contact irritability.[1]

§ 5. THE PHYSIOLOGY OF THE IRON-BACTERIA.

We are not yet in possession of a completely satisfactory explanation of this remarkable deposition of iron on the membranes of these bacteria. Cohn compared the deposition in Crenothrix polyspora to the deposition of silicon in diatoms. Zopf, who examined the same organism, declared that the deposition was purely mechanical, the iron being retained in the mucilaginous layer surrounding the cell in the same way that the coloured matter is retained by the gelatine in certain coloured jellies. Later, Winogradsky came to a quite different conclusion, maintaining that the bacteria derived some benefit, in the shape of an acquisition of energy, by the oxidation of the bicarbonate $FeH_2(CO_3)_2$ to the hydroxide $Fe_2(OH)_6$. These statements, however, were not supported by his researches on this matter, and Molisch's still later experiments show that a different explanation is more in accordance with the facts. This experimenter showed that these bacteria could be cultivated in solutions which did not contain a particle of iron. It was also demonstrated that the iron could be replaced by manganese with the same result, all the manganese being attracted to the membranes of the bacteria, just as the iron was. Further it is known that certain algae, *e.g.* Zygnema, have a power of attraction for certain metallic salts, *e.g.* compounds of chromium, aluminium, and iron, with the result that the alga becomes considerably swollen in appearance.

These experiments point to conclusions different from those come to by Winogradsky. They indicate that the accumulation of ferric hydroxide on the surface of these organisms is due rather to a purely chemiotactic power of attraction, than to a process vitally connected with the activities of the organisms, for on the one hand

[1] The author has lately published a preliminary notice of five new species of iron-bacteria, all of which were found in various samples of iron-water collected from different parts of Great Britain. See *Proc. Roy. Soc. Edinburgh*, Vol. XXVIII. Part V. No. 19.

the latter are able to thrive when no iron is present in the water, and on the other hand, oxidation into the red compound takes place quite readily when iron-bacteria are altogether absent. Hence the oxidation into the red compound takes place in the water, this compound being then attracted chemiotactically by the iron-bacteria if these be present. The deposition is not of a purely mechanical nature, for in many samples it is found that *all* the ferric hydroxide is located on the bacteria, not a particle being found in an unattached condition. Again, the deposition is selective, for when other organisms, algae, etc., are present in the iron-water, their surfaces are quite devoid of iron.

The accumulation of ferric hydroxide in water-pipes has on occasions been a source of great trouble, as has been amply shown by the experience of Berlin, Rotterdam, and other towns. Woltering and Sassen have recommended passing the drinking-water of a town through coke towers, before passing the water into the pipes. This would oxidise the iron, which would thus be precipitated before reaching the pipes. This method could not be used on a large scale, and it is doubtful whether even on a small scale it has been completely successful.

It has been suggested that iron-bacteria are mainly responsible for the decay of white sandstone, which affects so many buildings in this country that are built of this material. So far as the writer's observations go, there is no warrant for the assumption that the decay is due to bacterial action, and it is most certainly not due to the agency of any of the known iron-bacteria.

CHAPTER XII.

BACTERIA AND THE PRESERVATION OF FOOD-PRODUCTS.

§ 1. INTRODUCTION.

In dealing with this subject, the main fact to be borne in mind is that all organic material, whether animal or vegetable, is subject to the attacks of microorganisms. The dead animal or vegetable body becomes food-material for the living animal or plant. Now this food-material is strictly limited in amount and cannot be wasted; so re-constitution must be continually taking place. To the myriads of small organisms which are everywhere present, this important function has been relegated. If the trunk of a fallen tree be left exposed, after a number of years, it will have disappeared. Many millions of organisms, of many different kinds, will have fed on it. Those that came first will effect certain changes and then disappear, their place being taken by a different set of organisms. These again will leave their mark on the tree, and be succeeded by still other organisms, and so the endless series of changes goes on, till the tree finally disappears. The tree will become less and less, because gases and liquids will be formed as a result of these activities, and their loss naturally reduces the volume of the tree. These changes happen to all other organic substances, for if they ceased, owing to the strictly limited amount of the food-supply, all life would soon come to an end. When, therefore, an article of food turns "bad," it simply means that another step in the cycle of changes has been taken. Nature's laws are very drastic, and the order to "move on" cannot be evaded, though in a few cases, which we are about to consider, the order can be suspended for a short time.

Everything that we class as food-products is of an organic nature,

and under ordinary circumstances would soon suffer the changes that we have described above. These changes we call by such names as decay, putrefaction, rotting, etc. They are always initiated by micro-organisms (bacteria, moulds, yeasts, etc.), which are ever present in the atmosphere and in the soil, and only wait for favourable conditions to pounce upon their natural food. Our ability to prevent food-stuff from suffering these changes is due to our knowledge of the laws which regulate the growth and multiplication of these microorganisms. If, therefore, we can place the food-material under conditions so unfavourable that microorganisms cannot grow and multiply, we shall have achieved our object.

Now before putrefaction can take place the following conditions must hold, the absence of any one of them being sufficient to prevent this process setting in :

1. The microorganism must be present.
2. There must be an adequate supply of water.
3. The temperature must be suitable.
4. The food-stuff must not contain substances injurious to the growth of these small organisms.

With regard to the presence of microorganisms, as explained in a former chapter, there are very few places where they are absent, and it may safely be stated that there are no localities in which the preservation of food is carried on as an industrial occupation which are free from them. With regard to the second condition, no matter how suitable the food presented to them, bacteria cannot multiply when there is less than 30 per cent. of water, and, approximately, the same percentage holds for moulds, yeasts, etc. However, there are very few foods which in a normal condition hold such a small percentage of water, so that very few can escape on this score. Next, there are no places on the earth, even in the Persian Gulf, where the normal temperature is too high for bacterial and other growth, though for some distance from both the North and the South Poles, and on the tops of the highest mountains, the temperature is too low to permit of growth of any kind. Finally, there are no food-stuffs which naturally contain substances that are imperishable, and which, at the same time, prevent the growth of microorganisms. We therefore see that, though all four conditions must hold before putrefaction can set in, there is no hope of preservation except by artificial means. In adopting artificial means it is necessary to secure the absence of one condition only.

§ 2. FIRST METHOD OF FOOD-PRESERVATION—DESTRUCTION OF MICROORGANISMS.

We can take as our first method the various contrivances by which bacteria and kindred organisms are altogether eliminated from the food-stuff. The principle of the method consists in heating the food-material until all the organisms on and in it have been killed, then sealing it hermetically in sterilised cans. This method was invented by a French confectioner, named Appert, long before we knew anything about the rôle of bacteria. It is obvious that if no organisms are present there cannot be any decomposition. The method of procedure is well known. The food to be preserved is heated by being boiled in water or by the use of steam under pressure. When sufficiently boiled or steamed, the cans in which the food is placed are soldered up, thus effectually preventing the subsequent entrance of organisms from the atmosphere.

The development of this industry has materially affected the possibilities of agriculture in many countries. Formerly fruits that were very perishable had either to be dried or immediately consumed, so that small quantities only could be profitably grown. Now, however, the conditions are entirely changed, and large tracts of land in America and other places are devoted to the cultivation of very perishable fruits, like peaches and tomatoes. Not only fruits, but many other edible substances liable to rapid decay, *e.g.* different kinds of meats and edible parts of plants, are treated in the same way; so that there is nothing cultivated in any part of the world which cannot be brought to our doors in an unspoiled condition. The method, however, has its limits, for if the spores of bacteria be present in the food, mere heating or steaming is not sufficient to ensure their destruction, and if the spores subsequently germinate the food is ruined. We all know that not all the tinned food that comes to us from America is in perfect condition. Many cans of tomatoes, when opened, reveal only putrescent fruit, and occasionally the can itself is distorted owing to the pressure of the gases evolved during the putrefaction of the contained fruit. This state of affairs is usually due to the germination of various spores which have escaped destruction during the process of sealing. The germination of a single spore is sufficient to bring about the creation of a progeny of many millions, with disastrous results to the food. The putrefaction may, however, be due to secondary contamination, the result of infection by microorganisms between the time of heating

and the actual canning, or it may be the result of defective sealing. In whichever way it has resulted, it is always due to the multiplication of microorganisms which have not been eliminated from the food. From what has just been stated we must, in considering the adaptability of certain foods for canning, pay attention to the chances of the presence of these spores in the food. Tomatoes are more difficult to can than other fruits, owing to the greater frequency of the presence of species that form spores; the difficulty has, however, been largely overcome by slightly raising the temperature, and by somewhat prolonging the time of exposure to the heat. This results in the weakening or the death of the spores, thus greatly lessening the danger of putrefaction. By experience alone we can tell the degree of temperature, or the length of exposure which the fruit can stand without being appreciably deteriorated in value.

In America maize is now extensively canned, though for a long time the canning of this substance was thought to be impossible. This difficulty was overcome in the same way: it was found possible to make a slight increase in temperature, and give a longer heat exposure, without injuring the food.

Some time ago a sensation was caused in this country by the introduction of poisoned canned meat from America. This was probably not the result of defective canning, but rather the result of the use of poisoned meat for canning. The bacteria had done their work, leaving their poisons behind them, they themselves being either dead before reaching the factory or killed during the heating process. The meat was just as poisoned before being canned, as it was when the cans were opened in this country, for, as has already been explained, it does not directly matter whether the bacteria themselves are present or not, what does matter is the poison secretion of these organisms. In spite of these mishaps, however, there can be no doubt that the development of the canning industry has been a boon to mankind, and has vastly increased the agricultural resources of several countries.

§ 3. SECOND METHOD OF FOOD-PRESERVATION—DRYING.

This method consists in the removal of water, sufficiently to prevent the multiplication of the organisms, no attempt being made to kill them. No organism can grow if there is not a sufficient amount of water present. Living bacteria contain from 83 to 86 per cent. of

water. This may seem a very large percentage, but in comparison with other organisms, it is not an inordinate amount. Thus, human beings consist of 65-70 per cent., vegetables of 60-80 per cent., and algae of about 90 per cent. of water. It is small wonder, therefore, that drying should have such a marked effect upon the keeping qualities of various foods. This method is very old, and is the one most extensively employed, Thus, in the preparation of hay, the newly cut grass is left to dry, for the farmer is well aware that no putrefactive change can take place while the grass is in a dry state. In the case of flesh-foods drying is more difficult, because the process takes a long time, and during this period, in most countries, putrefaction would intervene before the process had been completed. In countries where the air is dry this process is quite feasible; thus, during the South African War, some of the raw flesh was prevented from putrefying for some time by direct exposure to the atmosphere; when thus exposed to the air no putrefaction set in. Many tribes of savages preserve meat by cutting it into thin strips and hanging it out in the sun to dry. The food called **pemmican** consists of meat cut into thin slices, divested of fat, and dried in the sun. After drying it is pounded, and then mixed with fat, dried fruit, etc. Pemmican will keep for a long time, and does not occupy much space.

The drying of flesh may be accomplished in another way, viz. by **smoking**. In this country smoking is carried on to a considerable extent. It must be noted that it is the drying and not the smoking that prevents bacteria and other organisms from gaining a footing, for though smoke is a slight antiseptic it is not powerful enough to prevent bacterial growth. In some places the smoke is obtained by the burning of certain woods, which contain antiseptic substances, like creosote and phenol. The evaporation of such substances add their quota to the injurious influences brought to bear on the bacteria.

The drying of fruits is at once a simple and an efficacious method of preservation, but many kinds of fruits, *e.g.* tomatoes, would be ruined during the process, so that drying is of no use in their case. Among others we may mention pears, apples, grapes, currants, raspberries, blackberries, figs, and dates, as fruits which readily lend themselves to preservation by the drying method. It is well to bear in mind that drying does not destroy the microorganisms on the fruit and it is only necessary to moisten the fruit and lay it aside for a day or two to observe putrefaction setting in: in a very short time the surface of the fruit will be covered with an extensive growth, usually of moulds, sometimes of other organisms. Of recent years, drying

by the use of hydraulic pressure to squeeze out the water is being successfully carried on.

The drying of burnt hay is an instance of another method of achieving the same end. This is done by what is called the "heat of fermentation." The grass is piled up in heaps about 12 feet high, then well trodden to prevent the admission of air. This results in a rapid rise of temperature, which is stopped when it reaches about 158° F., the stopping being effected by opening out the heaps and spreading the hay in thin layers on the ground. It usually takes from two to three days for the heaps to attain a temperature of 158° F. The heat in the hay is sufficient to cause a rapid drying, and after a single turning the hay is ready for storage. The rapid rise of temperature probably precludes the bacteria as agents in the development of this heat, which is in all likelihood produced by *oxidising ferments* which are present in the hay. These ferments decompose certain substances in the hay, the process resulting in a development of heat.

Still another example of the prevention of putrefaction by drying is shown by the method that is used in this country for the preservation of hay. The freshly-mown hay is spread out in the sun and dried. If rainy weather intervenes after the hay has been cut, the latter soon begins to change for the worse, but when dried and stacked it successfully resists the attacks of the organisms of putrefaction.

§ 4. THIRD METHOD OF FOOD-PRESERVATION—KEEPING THE TEMPERATURE LOW.

In this method preservation is effected by lowering the temperature to such a degree that the organisms scattered on and in the food-stuff find it impossible to multiply. As in the drying method, so here the mere lowering of the temperature does not kill the organisms, but merely prevents their multiplication. Thus, it has been found that Bacillus vulgaris and Bacillus coli communis were not killed even after an exposure of 10 hours to a temperature of −252° C. The vitality of the organisms on the frozen food will soon show itself by merely raising the temperature and keeping it raised for a few days. Meat at a low temperature is one of the staple imports of this country. It is not always in a good condition, but this is due to the quality of the meat being inferior, or to the meat being tainted before being subjected to freezing. When meat is kept a few degrees only above freezing-point, as is usually the case, it is in a condition in

which it can be attacked, though only mildly, by a few species of bacteria, to which the low temperature is only a partial hindrance. This chiefly accounts for the superiority of fresh over frozen meat.

With regard to fruits, although freezing ruins most of them, it is possible, by keeping the temperature a little above the freezing point, to preserve them for a long time without injury.

§ 5. FOURTH METHOD OF FOOD-PRESERVATION—CHEMICALS.

Great care must be exercised in the choice of the chemical that is to be used as a preservative, for it must possess the unusual property of being injurious to bacteria and similar organisms without being harmful to the human system. There are, rightly, stringent laws with regard to the use of these preservatives, for it would never do to allow the food-preserver to be the sole judge as to the proper preservative to make use of, for whilst he could ascertain what chemicals would prevent his food from becoming bad, he is not usually the right person to estimate their effects on the human system.

Fortunately there exist certain substances which, when present in small quantities, are beneficial or even necessary to a plant or an animal, but when present in larger amounts are highly prejudicial. We see this exemplified in the case of the scurvy attacks which, in the olden days, often afflicted sailors during long voyages. Their food was almost all salted, and the disease arose through having too much salt in their bodies. At the same time the human system cannot exist without a little salt. The same principle holds with regard to bacteria, so far as salt is concerned. In small quantities salt is generally added when nutrient media are being prepared for the cultivation of bacteria, but if more than a certain amount be used, it will have a prejudicial effect on the growth of the bacteria. If still further increased growth stops altogether. The value of salt as a preservative will therefore be readily perceived. We are all familiar with salted corned-beef, salted fish of various kinds, salt pork, and many other kinds of flesh that are prevented from decaying by the use of this valuable preservative.

Another preservative of the same nature is **sugar**, although there is no substance which bacteria and moulds of all kinds find so suitable as a food material. The same principle holds as in the case of salt, it is a food only when present in small quantities. An excess of sugar acts like an antiseptic. In making jams, advantage is taken of this fact,

and the difference in the amount of sugar is probably the reason why shop jam so seldom goes bad in comparison with the housewife's jam, and also why the latter has usually more of a fruity flavour. Condensed milk does not go bad, as would ordinary milk, because of the presence of a large quantity of sugar, and in the case of many dried fruits, *e.g.* raisins, the addition of sugar is employed as a supplement to the drying process in preserving these fruits.

Acetic acid in the form of vinegar is another substance which has a preserving value, though, unlike salt and sugar, it is not beneficial to bacteria, etc., even in small quantities. Its use is naturally limited on account of its acid nature. Vinegar is used instead of water in the preservation of vegetables by hermetical sealing. This is done in order to prevent **beet**, **gherkins**, and the like from losing their natural colour and to prevent decay; for vinegar is an antiseptic. **Mixed pickles**, for instance, are preserved in this way, and in some cases the boiling previous to sealing is omitted, as for instance in the pickling of gherkins.

In addition to these legitimate preservatives, mention must be made of those, the use of which as food-preservatives is forbidden by law. They usually appear in practice under concealed names. The commonest of such preservatives are boracic acid, borax, salicylic acid, and formalin. Boracic acid and borax are forbidden because of their injurious effects on the human system when *continually* consumed, whilst the other two are much more injurious.

§ 6. PRESERVATION OF EGGS.

The method of treatment of eggs for the purpose of preservation is somewhat peculiar, on account of the protecting shell with which the egg is covered. It might be imagined that such a protection would be sufficient to prevent bacteria and other organisms from gaining an entrance into the mass of the egg, but we know only too well that the keeping power of an untreated egg is not very great, in fact eggs go bad almost as quickly as if there were no protecting shell. This arises from the fact that the shell is porous, and does not prevent bacteria from getting in, and also from the fact that there are bacteria in the egg before the shell is deposited. It is obviously impossible to kill the bacteria that are already inside without at the same time ruining the egg, so what is usually done is to make the shell impervious to bacteria and to the oxygen of the atmosphere. This destroys the

possibility of multiplication of all the bacteria that are inside that need oxygen, and at the same time prevents others from getting in. The shell is made impervious by immersion in **milk of lime**, or much better, by the use of **water-glass**. The latter is a material composed of sodium and potassium silicate. It is cheap, and usually sold in the form of a thick syrup. One part of the syrup is dissolved in ten parts of water, the mixture being poured over the eggs. One gallon of this mixture is sufficient for the preservation of 50 dozen eggs. Eggs can be preserved from decay for several weeks, though indefinite preservation is quite out of the question, because some of the bacteria that are inside the shell, though very much handicapped, yet manage to multiply very slowly, and in course of time induce changes that materially alter the constitution of the egg.

CHAPTER XIII.

THE NITROGEN-BACTERIA.

§ 1. INTRODUCTION.

The relation of bacteria to nitrogen is the most important problem which presents itself to the agriculturist, the reason being that while nitrogen forms a very large proportion of the constituents necessary to build up a plant, it is present in the soil only in a limited quantity, and consequently, constant cropping soon exhausts the supply. To remedy this defect manuring in some form or other has to be resorted to. To ensure the growth of plants the presence of ten elements is necessary: carbon, hydrogen, oxygen, nitrogen, sulphur, phosphorus, potassium, calcium, iron, and magnesium. All of these, with the exception of nitrogen, phosphorus, and potassium, are present in abundance in the soil. Of these three, the amount of phosphorus and potassium which is required by plants is small, and an abundant supply is easily obtained. With nitrogen, however, the case is different. In the first place, the higher plants cannot assimilate this element unless it is presented to them in the form of nitrates. When, therefore, constant cropping has produced a dearth of available nitrogen in any given area, that area must be supplied either with nitrates or with some material like guano or dung which can be readily converted into nitrates by the soil-bacteria. But the supplies of manure we at present obtain from the deposits of nitre and guano, which nature has given us, cannot last much longer; so that some means must before long be adopted to supplement these supplies. Within the last twenty years the efforts of agricultural investigators have been attended with a large measure of success. It has been found that nature has means at her disposal for remedying the defect, and efforts are being made towards helping nature along her own lines. The importance of the fact that

bacteria are active agents in making good the loss of nitrogen cannot be overestimated. In this relation there are two distinct lines of discovery which must be treated separately, though we are dealing with only one class of organism. We have to deal with these nitrogen-organisms, first, when they are free in the soil, and second, when acting in conjunction with leguminous plants.

§ 2. THE ACTIVITIES OF THE NITROGEN-BACTERIA WHEN FREE IN THE SOIL.

Suppose a number of young dried leaves be collected from any forest tree, and then exposed to the atmosphere for a twelvemonth. At the end of that period it will be found that, though the total weight is less because some water has evaporated, yet the leaves have gained in nitrogen, in fact they will be found to contain twice as much nitrogen as they did before the exposure. In the same way the soil, when left untouched, will, under favourable conditions, accumulate nitrogen. The results of one experiment will make this clear. Fifty kilograms of earth were exposed for a certain time, and then analysed so far as its nitrogenous constituents were concerned.

Total 54·569	54·09 grams	of nitrogen were present in earth at beginning of experiment.	
	0·076 gram	,,	was gained through agency of rain.
	0·053 ,,	,,	was gained through agency of atmospheric ammonia.
	0·35 ,,	,,	was present in a few plants which had been inserted in the earth.
Total 59·178	56·54 grams	,,	were present at close of experiment.
	0·403 gram	,,	had been carried away by water.
	2·235 grams	,,	were present in the plants which had grown in the earth.

Hence the gain of nitrogen was 4·609 grams.

As this abstraction of nitrogen from the atmosphere is taking place wherever decomposition of this nature is taking place, it will be seen that a very large gain of nitrogen must annually accrue to the earth through this agency. When this process became known, attention was directed to the agents which Nature employed for this purpose. It was soon found that when the soil was sterilised no gain of nitrogen took place, so that the explanation of the gain could not be found in any chemical or physical action, but must be sought for in the

activities of organisms which are killed by sterilisation. In 1888 Beyrinck succeeded in making a pure culture of one of these nitrogen-fixing organisms. This one he called **Clostridium Pastorianum** (Fig. 105). He was able to cultivate it artificially, and the medium he employed was composed of the following substances:

Potassium hydrogen phosphate,	0·1%
Magnesium sulphate,	0·02%
Sodium chloride,	0·001–0·002%
Ferrous sulphate, Manganese sulphate,	traces
Dextrose,	2–4%
Water,	100 c.c

His method of "capture" was the same as is employed in making pure cultures from any mixture. A solution containing the above substances was inoculated with a small portion of soil. Owing to the nature of the medium the other soil bacteria were not able to effect multiplication. When growth had taken place, plate-cultures were made in the usual way, inoculations being taken from one of the colonies that had developed on the plates.

Fig. 105.—Clostridium Pastorianum.

Clostridium Pastorianum forms rods 1·2–1·3 μ thick and 1·5–2·0 μ long. It forms endospores and excretes butyric acid. The name "Clostridium" was given to it on account of the peculiar form which the individuals assume when they form endospores. When not forming spores the individuals cannot be distinguished from the members of the genus Bacillus. Another characteristic feature is that the action of iodine produces a peculiar violet-brown colouration of the individuals,

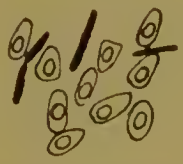


Fig. 106.—Clostridium Pastorianum. In sporogenous condition.

Fig. 107.—Clostridium Pastorianum. Germination of spore.

this also being a characteristic of the other butyric acid bacteria. This organism grows best when deprived of oxygen, though capable of growth when oxygen is present. The ripe spores are 1·6 μ long and 1·3 μ broad, and often lie in a roughly triangular covering (Fig. 106). The germination is polar (Fig. 107). In one experiment, in which a litre of nutrient solution containing no nitrogen was inoculated with

this microbe, it was found that after twenty days 53·6 milligrams of nitrogen had been taken from the atmosphere. In this particular experiment 40 grams of dextrose were present in the nutrient solution. At the close of the experiment all of this had disappeared, and its place had been taken by acetic and normal butyric acids, and traces of alcohol and lactic acid.

Beyrinck's brilliant researches also showed that Clostridium Pastorianum was not the only organism capable of assimilating the free nitrogen of the atmosphere.

By changing the form of the carbon supply in his nutrient solution, using, for example, mannite instead of dextrose, he was able to isolate from the soil two other similar organisms, which he named Azotobacter chroococcum and Azotobacter agilis respectively. These were found capable of assimilating the atmospheric nitrogen only when acting in symbiosis with other organisms. The genus Azotobacter is remarkable for the size of its cells, which resemble yeast more than bacteria cells. The general appearance of the individuals of this genus is given in Fig. 108. They appear as Diplococci or short rods 4-6 μ thick, have vacuolated contents, and shiny walls. They also possess short polar cilia, and are consequently normally motile. Judging from their shape, size, and general characteristics, it is still doubtful whether the genus Azotobacter should not be included among the lower Algae rather than in the Bacteriaceae. With regard to the Fungi, the following have been credited with possessing the power of assimilating free nitrogen from the atmosphere: *Aspergillus niger*, *Alternaria tenuis*, *Gymnoascus*, *Penicillium glaucum*, *Mucor stolonifer*, *Endococcus purpurascens*, *Phoma betae*. In most of these the amount of nitrogen gained was from 1 to 2 mgr. in a 50 c.c. nutrient solution. In Phoma betae, however, as much as 10·5 mgr. was obtained from the same quantity of nutrient solution. Unfortunately these results have not been fully confirmed, though they are probably correct. One subsequent research gave negative results, but it is possible that the Fungi do not always assimilate free nitrogen, but do so only when a certain set of conditions hold, so that we cannot throw over the positive results, but can only hold back our judgment till the results of further researches are forthcoming—a precaution which is rendered necessary when we remember how easily the physiological properties of bacteria can change.

Fig. 108.—Azotobacter.

With regard to the **Algae**, as far back as twenty years ago it was stated that they possessed this power of fixing nitrogen. The facts are as follows: If a plot of ground be cultivated with grass, potatoes or turnips, it does not gain nitrogen from the air if it be covered with sand to prevent the growth of Algae on it. On the other hand a gain does take place if Algae and Mosses are allowed to grow in the soil. Again, if a piece of ground be covered with an alga-vegetation, and another with a moss-vegetation, the former shows that nitrogen has been gained from the atmosphere, the latter that it has not. The conclusion that Algae can "fix" nitrogen must, however, be received with suspicion, as in the experiments care was not taken to remove the bacteria from the soil. It has been since shown that *no Algae in a pure cultivation can assimilate nitrogen*, but that many Algae in combination with earth bacteria can effect this fixation. It has therefore been suggested that they supply the carbohydrate material, got by them by assimilation, to the bacteria which effect the nitrogen fixation. In this way they may be regarded as helping to bring about the sum total of conditions which enable the bacteria to fix nitrogen, rather than as organisms that effect the fixation themselves. Finally, with regard to the *Higher Green Plants*, many researches have been made to determine their power of fixing nitrogen, but in all those in which positive results have been obtained, it has been found that the earth had not been sterilised. In all researches in which the earth had been sterilised negative results have invariably been obtained.

§ 3. CONDITIONS OF NITROGEN-FIXATION.

It is extremely important to the agriculturist to know the conditions which govern and regulate this nitrogen-fixation. Numerous researches have brought out the following results:

1. Of inorganic materials calcium and phosphorus are absolutely essential to the furtherance of fixation. Potassium and sodium seem to be unessential except in the case of Azotobacter, in which case the presence of one or both of these seems to help the fixation.

2. It is important that Algae should be present in the soil, for, as shown, the gain of nitrogen is considerably greater when they are present, because they probably supply the necessary carbon to the bacteria, which are thus able to fix nitrogen with greater energy than would otherwise be the case. It is not likely that any alga will serve for this purpose, and an alga that is favourable to, say, Azotobacter

may not be favourable to, say, Clostridium. The reader will readily see what a large amount of patient research is still necessary before we know all the possible combinations of these bacteria with algae which will produce the best results.

It was found, for instance, that whereas nitrogen-fixation resulted when either of the two algae, Cystococcus and Nostoc punctiforme, was added to a solution containing some earth-bacteria, this fixation did not take place when, instead, either of the two algae Schizothrix lardacea or Ulothrix flaccida was employed.

3. It is important to remember that the amount of fixation is closely governed by the amount of carbonaceous material supplied to the soil. This can be seen from the following table.

				Gain of Nitrogen.
1 litre nutrient solution contained	1	gram	Glucose,	7·4 mgr.
,, ,, ,,	2	,,		13·5 ,,
,, ,, ,,	3	,,		17·3 ,,
,, ,, ,,	4	,,		31·4 ,,
,, ,, ,,	5	,,		39·4 ,,
,, ,, ,,	6	,,		45·9 ,,
,, ,, ,,	7	,,		59·9 ,,
,, ,, ,,	10	,,		91·4 ,,
,, ,, ,,	12	,,		127·9 ,,
,, ,, ,,	15	,,		62·9 ,,

In this series the nitrogen-fixing organism was Azotobacter.

4. The process takes place in the dark as well as in the light, as these bacteria are to be found over 100 cms. under the surface.

5 It is agreed by all observers that the fixation of nitrogen is considerably hindered by the presence already of a large quantity of nitrogen compounds in the nutrient substance. But it has been affirmed that the presence of a *small* quantity of nitrogen is favourable rather than otherwise to the fixation of nitrogen.

6. With regard to **temperature**, it is stated that the process does not take place under 10° C., and not over 40°-50° C.

The increase in the nitrogen content of one kilogram of earth has been estimated to be as follows:

From May 29th to Oct. 10th, - - 0·0709–0·0933 gr.
,, Oct. 10th to Apr. 30th, - - 0·0933–0·0910 ,,
,, Apr. 30th to Oct. 10th, - - 0·0910–0·1179 ,,

7. The presence of much water in the soil is to be carefully avoided. When the percentage of water is more than 15, the fixation of nitrogen begins to suffer.

8. The earth should be aerated, as is shown by the following table, which gives the results of experiments carried out to show the effect of aeration and non-aeration at different depths in the soil.

Layer.	Aerated. Nit. present (%).	Non-aerated. Nit. present (%).
i. 1-20 cms.	0·132	0·113
ii. 20-40 cms.	0·109	0·074
iii. 40-60 cms.	0·076	0·059
iv. 60-89 cms.	0·069	0·046

The foregoing description shows that much has been done in this branch, and systematic nitrogen-farming of the atmosphere is almost within our reach. What is still lacking is an exact knowledge of all the factors which are detrimental, and all which are beneficial, to the growth of nitrogen-bacteria. We also require to know more of their relations to other organisms in the soil, for the struggle for existence is as keen here as in other places. It is probable that in a few years all these difficulties will disappear, for most of the important points bearing on the subject have already been mastered.

§ 4. NITROGEN-BACTERIA ACTING IN CONJUNCTION WITH LEGUMINOUS PLANTS.

There is another group of organisms of this class which have a most peculiar mode of life. They act in conjunction with plants belonging to the Leguminosae (Pea and Bean family). From very early times it was known that plants belonging to this family were able to thrive in soil which contained little or no available nitrogen, and that the soil became, in consequence of their growth, richer in nitrogen than it was before. A farmer does not, for example, sow wheat for two successive years in the same field. He follows wheat with clover, or some other leguminous plant, in order to make up for the deficiency of nitrogen in the soil which has been caused by cropping wheat. This is the principle upon which the *Rotation of Crops* is based. If the Leguminosae are thus so different from other plants, there must be some peculiarity in their nature to account for this remarkable difference. It had long been known that the roots of these plants have a swollen or nodular structure. In Lupinus the main root itself is swollen in appearance (Fig. 109), though usually it is the side roots that are nodular. A typical example is seen in Fig. 110, which shows the nodules of Robinia pseudacacia. Unfortunately these structures

were regarded as pathogenic until, in 1866, Woronin called attention to their cellular structure. Then they were more carefully studied, with the result that, in 1886, Hellriegel proved that there was a direct connection between the presence of these nodules and the power of nitrogen-assimilation from the atmosphere. From that time

FIG. 109.—Nodules on root of Lupin. (After A. Mayer.)

FIG. 110.—Nodules on root of Robinia pseudacacia. (After F. Nobbe.)

onward great strides have been made. It was ascertained that the nodules did not appear if the soil on which the leguminous plants were grown, was sterilised; so that the cause of the production of nodules had to be sought for among the organisms living in the soil. Finally, we owe to Beijerinck the credit of being the first, not only to identify this important organism, but also to isolate and cultivate it on artificial media. The organism turned out to be a member of the genus Bacillus, and the name *Bacillus radicicola* was henceforth assigned to it. The artificial medium used by Beijerinck was made up as follows:

Concoction of leaves of leguminous plants, -	100 c.c.
Gelatine. - - - - - - - -	7%
Asparagin, - - - - - - - -	¼%
Cane sugar, - - - - - - -	½%

The medium is finally made slightly acid.

The pure culture can be made in the following manner: A small portion of the contents of the bacteroid tissue is placed in a cubic centi-

metre of sterilised water, and a small quantity of the mixture transferred to a test-tube containing about 10 c.c. of the mixture mentioned above. Before the gelatine solidifies the contents of the test-tube are poured into a Petri-dish, which is then set aside until the colonies develop on the plate. It is absolutely necessary to see that no extraneous organisms obtain access to the mixture. To avoid this, the outside of the nodule must be cleaned first with water, then placed for a short time in alcohol, and finally in ether. When cut, all the usual precautions by sterilisation must be taken.

The colonies develop as small shiny spots which do not liquefy the gelatine. Once the colonies are developed they are transferred in the usual way to Agar-tubes, and thus can be studied as pure cultures.

§ 5. DESCRIPTION OF BACILLUS RADICICOLA.

These microbes are found as either motile or immotile rods. The latter are about 1 μ in breadth and 4–5 μ in length, whereas the former are much smaller, being only 0·18 μ in breadth and 0·9 μ in length. This diversity in size and motility is one of many instances of the multiplicity of morphological and physiological characteristics assumed by one and the same species. These are also strongly aerobic, and are killed when the temperature reaches 60-70° C.

It is doubtful whether all the bacteria to be found in leguminous nodules belong to one species; and much controversy has arisen on this subject. In the work of recent observers many varieties of this species have been obtained. This of course is only what is to be expected when the conditions of growth are so varied and bacteria so plastic. It has not yet, however, been indubitably proved that these varieties belong to more than one species.

Side by side with these researches on the nature of the bacteria, much light has been thrown on the effect of the presence of the nodules on the growth of the plants. It has already been mentioned that if the soil be sterilised no nodules are produced on the plants, and the same result is obtained with water cultures of these plants. These experiments were extended until the following facts, established chiefly by Hellriegel, were placed beyond doubt.

1. Leguminous plants, so far as nitrogen is concerned, behave in a different way to grass plants.

2. Grass plants obtain their nitrogen entirely from the soil, and their development has a direct ratio to the amount of nitrogen in the soil.

3. Leguminous plants obtain nitrogen not only from the soil, but also, to a far greater extent, from the atmosphere.

4. Leguminous plants cannot, unaided, obtain nitrogen from the atmosphere, but can do so with the help of bacteria which live in the soil.

5. These bacteria enter into a symbiotic relation with leguminous plants, and that relationship results in the formation of nodules.

6. The nodules are normal structures, which are absolutely necessary before this symbiotic relation can take place.

As one interesting example of the experiments which led to a knowledge of these important facts, the following is instructive.

A number of zinc boxes were each filled with 24 kilogr. of washed calcareous river-sand devoid of nitrogen. After the addition of the necessary mineral constituents, seeds of the plants mentioned in the table were sown. Some of the boxes received in addition each 0·83 gram of nitrogen in the form of nitre. The total dry matter and the total amount of nitrogen in the crop were then estimated, with the following results:

Per Box.	Oats.		Buckwheat.		Rape.		Peas.		Vetches.	
	Without N.	With N.	Without N.	With N.	Without N.	With N.	Without N.	With N.	Without N.	With N.
	Grams.	Grams.	Grams.	Grams.	Grams.	Grams.	Grams.	Grams.	Grams.	Grams.
Dry matter in crop, - -	24	91	12	44	13	50	352	330	250	241
Nitrogen in the the crop, - -	0·15	0·44	0·14	0·43	0·13	0·50	6·74	6·45	6·01	5·95

An examination of this table shows that in the non-leguminous plants the nitrogen in the crop is less in quantity than the amount present in the seeds, or in the seeds plus the added 0·83 gram. The inference, therefore, is that the nitrogen registered in the crop of these plants has all been derived either from the seeds or from the added nitre. In the leguminous plants, on the contrary, the nitrogen in the crop is much greater than the total amount of nitrogen which was present in the seeds and the nitre. The only way of accounting for this increase is by assuming that the nitrogen was derived from the atmosphere.

§ 6. MODE OF ENTRANCE OF BACTERIA INTO LEGUMINOUS PLANTS.

As the bacteria are not present in the seeds, they must gain entrance to the roots of leguminous plants from the surrounding soil. This is effected, in some at least of these plants, in the following manner: The end of a root-hair changes its form and becomes more or less sickle-shaped. The next stage consists in the formation on the inside of the wall of the root-hair of a shiny colony of bacteria, which then sends out a tube filled with bacteria. This tube (Fig. 111)

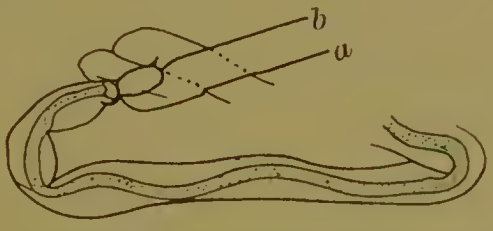


FIG. 111.—To illustrate mode of entrance of nitrogen-bacteria into roots of leguminous plants. (For explanation see text.)
a, Bacterial tube consisting of millions of bacteria; *b*, Root-hair. (After Nobbe.)

branches and works its way into the inside of the root, ultimately causing hypertrophy of the cells, and the formation of the *bacteroid tissue*. This method of formation, however, has been denied by other writers. It has been stated that it is the plant itself, and not the bacteria, that gives rise to the tube—known as the *infection thread*—because it is claimed that it has a structure which could be formed only from the protoplasm of higher plants, that is, that its walls are composed of a substance allied to cellulose which is not formed in bacterial cells. This point is not yet settled, but it is highly probable that, as the leguminous plant is ultimately parasitic on the bacteria, and derives such immense benefit from their presence, the latter explanation is the correct one.

The structure and course of the infection threads can best be ascertained by staining sections with a liquid made up by dissolving equal quantities of fuchsin and methyl-violet in 1 per cent. acetic acid. The protoplasmic contents and the membrane of the bacteroid tissue are coloured blue, the bacteria are coloured red, whilst the wall of the infection thread remains uncoloured.

The Bacteroids. The bacteria inside the bacteroid tissue undergo strange changes of form, as can be seen in Fig. 112, where some of the more noticeable forms are presented. It must be noted that

distorted forms such as these are common in many bacterial cultures when the conditions of growth are not favourable. They are *involution* structures, and are either already devoid of life or are approaching

Fig. 112.—Bac. radicicola. Showing involution-forms. (After Lafar.)

that state. Formerly all the bacteria inside the bacteroid tissue were denominated 'bacteroids,' but it is now customary to restrict this term only to the involution structures that are abundantly found in this tissue.

§ 7. RELATION OF THE BACTERIA TO LEGUMINOUS PLANTS.

It was formerly held that the relation was of a symbiotic nature, in which the plant supplied the necessary carbonaceous material, which was repaid by the microbe by a delivery of nitrogenous substances. That doctrine, however, in the light of the results obtained by Hiltner and other recent workers, cannot be regarded as established. The real nature of the relationship may be seen by a consideration of the following facts. In the first place, bacteria, taken from, say, pea nodules and used to inoculate a bean plant do not produce nodules, whereas if inoculated into a pea plant, they do. Now it is comparatively easy by gradual adaptation, to enable, for example, bacteria from pea nodules to grow in bean plants. The relation, therefore, is not a deep-seated one, and either organism can exist without the other. Again, the behaviour of these microbes is not what would be expected from a symbiont. If the inoculating material be weak the bacteria enter the roots, but remove themselves

again, being apparently too weak to maintain their entrance. This happens before they have formed nodules. If the inoculating material be somewhat stronger, the bacteria may be successful in their attempts at nodule formation. But no nitrogen-assimilation takes place if the cell-nuclei of the bacteroid tissue are able to offer a sufficiently strong resistance. A still stronger inoculating liquid overcomes this resistance and nitrogen-assimilation results. This resistance of the plant indicates that the bacteria are rather of a parasitic than of a symbiotic nature. This is strengthened by the fact that a weakening of the plant after the entrance of the bacteria, results in a total absence of nitrogen assimilation, the bacteria acting as pure parasites. On the other hand, if no weakening of the plant takes place, it is able, subsequently, to turn round on its oppressor, and abstract nitrogen from it, that is to say, the plant becomes parasitic on the bacteria, large numbers of which then assume the bacteroid form. We have therefore what may be called a case of *alternate parasitism*. The bacteria enter as parasites. If the plant be weak they remain such, but if the plant can sustain the attack, a counter-attack is usually successfully made.

§ 8. INOCULATION OF THE SOIL WITH BACILLUS RADICICOLA.

Obviously it will be advantageous to leguminous plants if the soil on which it is proposed to grow them, be well stocked with Bacillus radicicola. But to attain that end it is necessary to do more than merely bestrew the ground with cultures of that organism. We must take into account the conditions affecting the well-being of the bacteria as well as those affecting the well-being of the leguminous plants. In the first place, the soil must not be too acid, neither must it be deficient in lime, potash, or phosphates. Again, the soil must not be too rich in nitrates, for experience has shown that the leguminous plants prefer their nitrogen in this form, if plentiful enough, and consequently in rich soils bestrewing the ground with solutions containing Bac. radicicola has not always led to satisfactory results. Further, it is not sufficient to use any variety of Bacillus radicicola. If, for example, the soil on which peas are to be grown be inoculated with a variety taken from bean nodules the nodular production will probably not be great, so a variety must be used that is accustomed to pea plants. In many cases even this has not been found sufficient

to produce satisfactory nodules, because the culture of bacteria that was used was not *virulent* enough. The want of virulence is due to the fact that the species has been grown for several generations in an artificial medium, and that kind of growth has caused it to change its properties. The pure cultures of this bacillus called "Nitragin" which have appeared on the market have not until recently been an unqualified success, owing to want of appreciation of the selective nature of each variety of Bac. radicicola, beans, for instance, being inoculated with a variety taken from peas, and so on. Mention must also be made of another disadvantage which, like the others, has been overcome. In cultivated soils the seed in swelling, previous to germination, appears to be able to secrete a substance which tends to prevent the formation of nodules. This difficulty was overcome by adding to the solution containing the bacteria 1–2 per cent. of grape sugar and peptone.

When all these factors are taken into consideration, success has almost invariably resulted. In the cultures sent out, with careful instructions, from the Botanical Laboratory of King's College, London, over 80 per cent. of the experiments were successful, and Prof. Bottomley reports that he was able to grow a fine crop of Mexican beans on volcanic ash by simply adding culture solution to the ash. A couple of examples will be sufficient to indicate the gain derived from scientific inoculation. Tares were grown in sterilised soil. One set of pots received the necessary amount of nitrate of soda; another set, receiving no nitrate of soda, was inoculated with these bacteria. At the end of the season analysis gave the following results:

	Percentage of Nitrogen.
Tares manured with nitrate of soda,	1·92
Tares inoculated,	3·07

In Kilmarnock, field experiments with lucerne resulted as follows:

	Percentage of Nitrogen.
Section A, no nitrogenous manure,	3·41
„ B, nitrate of soda added,	3·75
„ C, inoculated,	4·04

CHAPTER XIV.

NITRIFICATION AND DENITRIFICATION.

§ 1. NITRIFICATION.

One of the results of the decomposition of the dead organic matter which is found in the soil is the production of ammonia gas (NH_3). A portion of this escapes into the atmosphere, and it is the liberation of this gas which is responsible for the sharp acrid smell which often accompanies decomposition. A large part of the ammonia, however, combines with the sulphuric acid that is almost certain to be present in the soil, forming with it the compound *ammonium sulphate*, which in turn is partially or wholly changed into *ammonium carbonate*. This last-named substance, not being volatile, does not escape into the atmosphere. Now, although a limited number of plants are able to absorb ammonium carbonate, and utilise the nitrogen contained in it as food-material, the majority of plants cannot make use of the nitrogen when combined in this form. Hence it would be a serious matter if the nitrogen were allowed to remain in this inaccessible condition.

In nature, however, the bulk of the ammonium carbonate is changed into **nitrate**, in which form the contained nitrogen is accessible to the majority of plants. The change from the ammonium compound into the nitrate is called **nitrification**. Until comparatively recent times it was supposed that this was accomplished by purely chemical means, for it was known that when nitrogenous organic material was brought into intimate contact with oxygen, as, for instance, through the agency of platinum-black, oxidation took place, and by this means it is possible to transform ammonium into nitrate compounds. This may be represented as follows:

$$\underset{\text{ammonia}}{2NH_3} + \underset{\text{oxygen}}{3O_2} = \underset{\text{nitrous acid}}{2HNO_2} + \underset{\text{water}}{2H_2O}.$$

This, it will be observed, is a process of **oxidation**, inasmuch as oxygen is absorbed in order to effect the chemical change. By a further oxidation the nitrous acid can be changed into **nitric acid**:

$$\underset{\text{nitrous acid}}{HNO_2} + \underset{\text{oxygen}}{O} = \underset{\text{nitric acid}}{HNO_3}.$$

What applies to ammonia applies to ammonium salts, in which case the oxidation results in salts of nitric acid, *i.e.* nitrates.

The oxidation of ammonium compounds in the soil was formerly regarded as a purely chemical process, the oxygen being supplied from the atmosphere. This was apparently borne out by the fact that it was not possible to secure adequate nitrification in a soil unless the latter were thoroughly aerated. However, Pasteur's epoch-making researches, demonstrating the intimate connection between micro-organisms and many of the processes that take place in nature, resulted in a closer study of nitrification from this point of view, and in 1877 it was shown that if a plot of soil were sterilised by heat, or by some other means, no nitrification took place. Now, if nitrification were a purely chemical process, the mere sterilising of the soil should not have stopped that process. Hence it was concluded that sterilisation of the soil had killed off the microorganisms which were concerned in carrying on this process of oxidation. The next step was obviously to seek out these important organisms among the many thousands that are found in the soil. This proved a difficult task. Whilst a portion of soil placed in an ammoniacal nutrient solution invariably oxidised the ammonia into nitrites and the latter into nitrates, none of the soil bacteria that appeared on the gelatine plates, used for the purpose of isolating these bacteria, were able to accomplish this. It was evident, therefore, that they did not grow in the ordinary nutrient media. The difficulty was at last surmounted by Winogradsky in 1889, and also independently about the same time by Paul G. Frankland in England and by Jordan and Richards in America. Winogradsky effected the isolation of the first of these organisms *by employing a nutrient medium which did not contain a particle of organic matter*. It was made up as follows:

Ammonium sulphate, - - - - -	1 gram
Potassium phosphate, - - - - -	1 ,,
Tap water, - - - - - - -	1 litre.

In addition to every 100 c.c. of the nutrient medium, from 0·5 to 1·0 gram of basic magnesium carbonate was added. The absence of organic matter prevented all except a very small number of organisms

from growing, and among these the nitrification bacteria were predominant. By an ingenious device the Russian bacteriologist succeeded in effecting a pure culture, so another step in our knowledge of this subject was achieved. Now, when this organism was inoculated into a sterilised ammoniacal nutrient solution, it was found that it was capable only of changing ammonia into nitrite; no subsequent change of the latter into a nitrate took place when pure cultures were used. The discovery of this fact showed that the process of nitrification is not performed wholly by one kind of organism, there must also be present in the soil another kind which changes the nitrite into nitrate. It was not long before the genius of Winogradsky enabled him to isolate from the soil an organism which effected this change, so that now the essentials of the whole process can be regarded as complete. These bacterial organisms isolated by Winogradsky and others may all be classified under one or other of the two heads: the **nitrite-bacteria** which oxidise ammonium compounds into nitrites, and **nitrate-bacteria** which still further oxidise the nitrites into nitrates.

§ 2. THE NITRITE-BACTERIA.

The first of the nitrite-bacteria was isolated by Winogradsky from a specimen of soil which came from Zurich (Switzerland). This organism was named first **Nitromonas**, this being subsequently changed into **Nitrosomonas**. It is important to note that the name applies not so much to a single species as to a group of very closely allied organisms, the differences between which being so small that it is not advisable to give each a separate name. The variety found at Zurich is very widely distributed, and is practically the only one of this kind that is found in West Europe. The individuals are extremely small, and do not create turbidity in the culture fluid, as is done by the majority of bacteria. In fact they are not usually found in the liquid, but rather in the sediment as compact Zoogloeae of various sizes, each of the latter being round, and measuring from 10 μ to 15 μ in diameter (Fig. 113). In order to see the individuals which are embedded in a Zoogloea, careful staining is necessary, the best for the purpose being a solution of iodine in potassium iodide. A 7–10 days' old culture shows, when undisturbed, either a very

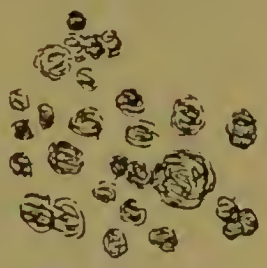


Fig. 113.—Zoogloeae from nitrite-bacteria. (Zurich.)

slight turbidity just above the sediment of the culture fluid, or at the same place, a strong opalescence. A microscopical examination reveals not only Zoogloea masses but also free-swimming individuals and all stages of intermediate conditions. If examined a day or two later, when all the ammonia of the culture fluid will have been used up, it will be found that the motility has been lost, and all the individuals will have sunk into the sediment at the bottom of the culture fluid. Fig. 114 shows the microscopic appearance presented by the free-swimming individuals. The form of the individuals, as seen in the figure, is like the letter O, the width being 0·9–1·0 μ and the length 1·2–1·8 μ.

Fig. 114.—Nitrite-bacteria. (Zurich.) Free-swimming individuals.

Motility is effected by the activity of one somewhat elongated cilium attached to one of the ends. The greater the predominance of the free motile individuals over those embedded in the Zoogloeae the greater is the capacity for oxidation possessed by the culture, and, it follows, the quicker is the rate of transformation of the ammonia-compound into the nitrite. When the ordinary nutrient media, bouillon, nutrient agar, nutrient gelatine, etc., are inoculated with these bacteria, no trace of growth is observed. As will be presently seen, these organisms derive their nitrogen from the ammonia compound, and their carbon from the carbon dioxide of the atmosphere.

With regard to the nutrient solutions for the cultivation of these bacteria, the best results have been got from the following:

(1)

Ammonium sulphate,	1 gram
Potassium phosphate,	1 ,,
Well-water,	1 litre

Basic magnesium sulphate in excess.

or

(2)

Ammonium sulphate,	1 gram
Potassium phosphate,	1 ,,
Magnesium sulphate,	0·5 ,,
Sodium chloride,	2 grams
Ferrous oxide,	0·4 gram
Distilled water,	1 litre

Basic magnesium sulphate in excess.

or (3)

Ammonium sulphate,	2–2·5 grams
Potassium phosphate,	1 gram
Magnesium sulphate,	0·5 ,,
Calcium chloride,	trace
Distilled water,	1 litre

Basic magnesium sulphate in excess.

(About 1 grain of basic magnesium sulphate per $\frac{1}{16}$ gram of ammonium sulphate has been recommended.)

Beijerinck gives the following prescription for the preparation of agar which will not inhibit the growth of these organisms. The agar is dissolved in distilled water, filtered, and then allowed to remain as a solid layer under water for two weeks. This sets up putrefaction in the agar, the superimposed water becomes turbid, and a somewhat nasty odour is emitted. This water is poured off and replaced by a fresh supply. After another two weeks the agar will have been sufficiently purified for the purpose. To it are added 0·2 per cent. of sodium ammonium phosphate, 0·05 per cent. of calcium chloride, and 0·05 per cent. of chalk. On the plates of the nutrient agar prepared in this way colonies do not appear for three or four weeks after inoculation. A quicker growth can be obtained by using Winogradsky's *Silicon jelly* as the solidifying medium. The preparation of the nutrient medium of which silicon jelly forms a part is somewhat complicated, and we cannot enter into the details here, but in its composition are also found the following substances: ammonium sulphate, potassium phosphate, magnesium sulphate, ferrous sulphate, sodium chloride, and magnesium carbonate.[1]

§ 3. VARIETIES OF NITRITE-BACTERIA.

It has already been stated that the first of these bacteria was found in Zurich. The same variety has been isolated from the neighbourhood of Paris, and has since been found to be quite common over the whole of West Europe. The variety that was found in St. Petersburg differs from the above in being quite round and somewhat smaller, being about 1 μ in diameter and so far as yet observed immotile. Otherwise the life history of this variety is identical with that of the above-mentioned forms.

A variety of the same kind of bacteria is a small coccus 0·5–0·6 μ in diameter, and provided with a cilium 30 μ in length. The life-history of this organism, which was obtained from Java, agrees in all respects with that of the European varieties, though found at the other side of the globe. Fig. 115 shows a Zoogloea mass of this variety. This has an unusually thick texture; so much so that the contained individuals cannot be distinguished even with the aid of stains. The free individuals in the motile condition possess each a long cilium, which causes the individual to exhibit a slow, hovering

[1] A detailed method of preparation of this nutrient medium is given in Lafar's *Handbuch der technischen Mycologie*. Vierte Lieferung.

kind of movement (Fig. 116). When the motile individuals come to rest they collect together to form irregular angular colonies, which gradually collect to form the Zoogloeae. A variety isolated from Tokio (Japan) was also found to exhibit the same characteristics as the above varieties, and with the exception of small differences

Fig. 115.—Zoogloea of nitrite-bacteria.

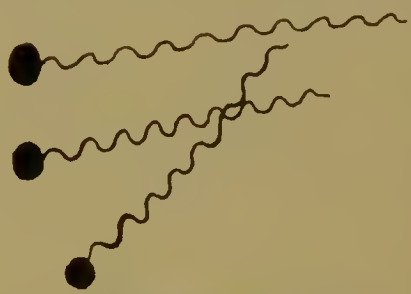


Fig. 116.—Nitrite-bacteria. (Java.) Free-swimming individuals.

with regard to the size of the individuals and of the Zoogloeae, the same can be said of the varieties obtained from Algiers and Tunis (North Africa).

The greatest deviation from the other varieties was found in a nitrite microbe which was isolated from Quito (South America). In this variety the individuals are round, being about 1·5–1·7 μ in diameter, and are remarkable in never passing over into the Zoogloea condition, always remaining free and motile. On silicon jelly relatively large colonies are formed, each of which has the appearance of a drop of turbid, yellowish liquid. Closely related to the Quito variety is one isolated from Brazil, though the individuals of the latter are larger, extending to about 2 μ or more. Finally, an Australian variety has been examined and found to agree with the South American varieties in almost all particulars, the chief difference being the smallness of the individuals of the Australian variety.

Although a good deal still remains to be done with regard to the differences and similarities of the various varieties of the nitrite-bacteria, as seen from the above account, all are very closely connected, and it seems probable that all have been derived originally from a single species, that has spread itself practically over the whole globe.

§ 4. PHYSIOLOGICAL CHARACTERISTICS OF THE NITRITE-BACTERIA.

These bacteria have a remarkable power of assimilating the atmospheric carbon dioxide and utilizing it as a source of food. This power is extremely interesting, because its discovery led to the overthrow of the doctrine that had long been held by botanists, namely that only plants possessing the green colouring matter *chlorophyll*, and these only in the daylight, were able to assimilate carbon dioxide from the atmosphere. Here, however, organisms were found that could do this without the aid either of chlorophyll or of light. Scarcely less interesting is their relation to organic matter. If any organic matter be present in their nutrient medium, even in very small quantities, growth cannot take place; that is, the addition of organic substances has the same effect as the addition of antiseptics. Indeed, it is remarkable that those organic substances that are most readily assimilable by other bacteria are the most injurious in their effects on the nitrite-bacteria. This applies particularly to *glucose* and *peptone*, which are introduced into almost every medium that is prepared for the nourishment of bacteria. The presence even of 0·025 per cent. of either of these two substances has an injurious effect, and 0·2 per cent. is sufficient to arrest growth altogether. These bacteria must therefore derive their carbon and their nitrogen from inorganic materials. Their carbon is obtained, as mentioned above, from the carbon dioxide of the atmosphere, whilst their nitrogen is got from the ammonium compounds which are always present in the soils, etc., in which these bacteria are found.

To secure the multiplication of nitrite-bacteria it is absolutely necessary to supply them with some ammonium compound. Among the various salts of that base, ammonium sulphate gives the best results.

As a general rule in bacterial cultures, the presence of substances produced by the activities of any particular microbe—*i.e.* waste products—is injurious to its further growth. That rule applies in the case of the nitrite-bacteria. Hence the more nitrites that are present the less active is the growth of the nitrite-bacteria. It is curious to note that the introduction of a small quantity of a nitrite into a culture fluid before inoculation with nitrite-bacteria has a far greater effect in preventing their growth than if the same quantity is introduced, after growth has well started.

Another characteristic which it is important to bear in mind when cultivating these organisms is their need of an abundant supply of oxygen. In Winogradsky's experiments most of the culture flasks had flat bottoms 12 cms. in diameter, and the height of the culture fluid was not allowed to reach more than 1 cm. high, in order to secure an adequate supply of oxygen. Several contrivances have lately been described which accomplish the same purpose in a more elaborate but more effective way. Of these, mention may be made of two, viz. one in which the free access of air is secured by placing the culture fluid in a rotatory apparatus, and the other in which this fluid is placed in a flask filled with some very porous material like slag.

§ 5. THE NITRATE-BACTERIA.

As already mentioned, the first of the nitrate-bacteria was discovered by Winogradsky in 1891. We have already stated the circumstances which led to the search for these bacteria. A sample of soil from Quito (S. America) yielded the first of them, and nitrate-bacteria have since been isolated from Russia, Germany, Great Britain, and other European countries. As was the case with the nitrite-bacteria, all these proved to be so much alike that all of them have been included under one species.

To cultivate these bacteria the following nutrient medium is generally employed:

Sodium nitrite,	1·0 gram
Potassium phosphate,	0·5 „
Magnesium sulphate,	0·3 „
Soda (free from water),	1·0 „
Sodium chloride,	0·5 „
Ferrous sulphate,	0·4 „
Distilled water,	1 litre

If a solid medium be required, the medium is made up as follows:

Sodium nitrite,	2 grams
Soda (free from water),	1 gram
Potassium phosphate,	tiny amount
Agar,	15 grams
Tap water,	1 litre

It will be noticed that these prescriptions, like those for the cultivation of the nitrite-bacteria, are devoid of organic substances.

§ 6. MORPHOLOGICAL CHARACTERISTICS OF THE NITRATE-BACTERIA.

The colonies on agar plates take a long time to grow, as they are not visible, even as small specks, till about two weeks after inoculation. Then round, homogeneous colonies, which may attain a diameter of from 100 μ to 180 μ, gradually develop. The individuals composing the colonies are rod-shaped, and very small, being about 1 μ long and 0·3–0·4 μ broad (Fig. 117). They have not been observed to exhibit motility, neither do they unite to form the Zoogloeae which are so characteristic of the nitrite-bacteria.

Fig. 117.—Nitrate-bacteria.

PHYSIOLOGICAL CHARACTERISTICS OF THE NITRATE-BACTERIA.

The statements that have been made above with regard to the physiological characteristics of the nitrite-bacteria apply equally to the nitrate-bacteria, for they also can assimilate the atmospheric carbon dioxide without the aid either of chlorophyll or of light; and further, they thrive only in media from which organic substances have been carefully removed. In the same way also, the presence of organic substances is detrimental to the growth of the nitrate-bacteria, though they are not so sensitive in this respect as are the nitrite-bacteria.

They differ from the nitrite-bacteria in that they obtain their nitrogen supply from nitrite- instead of from ammonium-compounds, hence they take up a substance which has been excreted by the nitrite bacteria.

As explained above, the nitrites are oxidised into nitrates as a result of passing through the bodies of the nitrate-bacteria. We have seen that ammonium compounds are indispensable to the nitrite-bacteria. To the nitrate-bacteria, however, the presence of an ammonium compound is unfavourable to growth. In a mixed culture containing ammonia the development of the nitrate-bacteria, if present, is arrested until all the ammonia has been used up. Among the different nitrite salts, that of sodium is best suited for the needs of these bacteria, and it is in this form that the nitrite is usually supplied.

In the case of the nitrate-bacteria we get an illustration of an exception to the general rule that the waste products of an organism tend to retard its further development, for the nitrates which are the chief products of their activity are not injurious to the nitrate-bacteria. Hence, if the conditions are otherwise favourable, they can transform into nitrates the whole of the nitrites presented to them.

Finally, the nitrate-bacteria are very strongly aerobic, and consequently all the special contrivances to secure an abundant supply of oxygen mentioned in connection with the nitrite-bacteria apply equally to these organisms.

§ 7. NITRIFICATION IN NATURE.

By nitrification is meant the gradual change of the ammonium salts of the soil into the corresponding nitrate salts.

The effect of this process is well exhibited in the saltpetre beds of South America, in places where sewage matter is being disposed of, and generally in all places where the exertions of the saprophytic bacteria have resulted in the production of ammonium compounds. As is to be expected from such strongly aerobic organisms, nitrite- and nitrate-bacteria are to be found only on and near the surface. Consequently we find them in greatest number in the first six inches of soil; below this depth their numbers rapidly decrease, and at a depth of two feet they are altogether absent. As they are very sensitive to drought, they are altogether absent from soils that have been dried up for some time. For experimental purposes, therefore, care is taken to use soil that is still somewhat moist. Forest soil is destitute of these bacteria: this is probably accounted for by the inhibitory effects, produced by the presence of a large amount of organic matter which is generally found in such soils. A chemical analysis of a soil that is undergoing nitrification shows no trace, or only small traces, of nitrites, and until this was explained doubts existed in the minds of many as to the validity of the bacteriological explanation of nitrification. A set of experiments, instituted by Omelianski, when supported by all the investigations which have been described in the foregoing paragraphs, put all these doubts to rest. Into a dilute bouillon culture fluid the following combination was introduced: Bac. ramosus (*A*), an ammonia producer, + a nitrite-producing microbe (*B*), + a nitrate-producing microbe (*C*). After 3-4 days ammonia was found to have been formed; after 7 days a nitrite reaction was demonstrated, and a

month later the nitrite had all disappeared, its place being taken by the nitrate. The combination $A + B$ produced in a similar culture fluid first ammonia, and later nitrite, but no trace of nitrate. Finally, the combination $B + C$ did not effect a change of any kind, even after 10 months. In these experiments, if any further proofs were required, we have a proof both of the validity of the bacteriological explanation of nitrification and also of the serial nature of the process. In nature the absence of ammonium compounds prevents the growth of nitrite-bacteria, and the absence of nitrites that of the nitrate-bacteria. The formation of ammonium compounds, and the consequent diminution of the organic material, due to the activity of the saprophytic bacteria, enables the nitrite-bacteria to multiply. Their multiplication results in the using up of the ammonium compounds and in the accumulation of nitrites, both of which conditions are unfavourable to the continued multiplication of the nitrite-bacteria. But these conditions are exceedingly favourable to the development of the nitrate-bacteria, which consequently predominate, and transform the nitrites into nitrates.

§ 8. ORGANISMS THAT REDUCE NITROGEN COMPOUNDS: DENITRIFICATION.

So far in this chapter we have been dealing with organisms that promote the oxidation of the nitrogenous material of the soil, thus rendering it suitable for assimilation by the higher plants. We must now devote a small space to those bacteria that work in an exactly opposite direction, removing the oxygen from nitrogenous compounds, and thus rendering them useless to the higher plants. Some of these organisms take away a part of the oxygen from the nitrates, converting them into nitrites, or they may take away the whole of this element, reducing them to ammonia. Other organisms of this class convert the nitrates and nitrites into nitrous oxide (N_2O) and nitric oxide (NO), both of which are also useless to the higher plants. Finally, a class of organisms exists which detach the nitrogen altogether from its compounds, when it escapes into the atmosphere as free nitrogen. The last process is termed **denitrification**. The power of reducing nitrates into nitrites and ammonia is possessed by a very large number of bacteria, though these are not dependent on this process for their existence, as they thrive very well when nitrates are altogether absent from the media in which they are cultivated. Frankland states that

out of 32 species of bacteria examined by him 16 or 17 possessed this reducing power, and of the 25 species examined by Warington no less than 18 were found to have this power. Other observers have fully confirmed these results. The list of these bacteria includes a number of well known saprophytes and parasites, *e.g.* Bacillus coli communis, Bac. fluorescens non-liquefaciens, and the microbes of cholera and typhus. It is evident from the diversity of these organisms that the power of reduction, under special circumstances, is possessed by a very large number of bacteria, this power being in some way advantageous to the organism exercising it. In fact it seems certain that this reduction is undertaken in order to secure the oxygen for purposes of bacterial respiration. That is to say, oxygen is made use of to break up organic matter so that the bacteria may utilise the energy that is thus liberated.

Another kind of reduction which is effected by some microorganisms is the change of nitrates and nitrites into nitrous and nitric oxides. This was observed in connection with the tobacco industry as early as 1868. If saltpetre and sugar are introduced into a culture fluid which has been inoculated with a portion of soil it is very common to find that the saltpetre has partially disappeared, its place being taken by nitrous and nitric oxides. That this is accomplished by the microorganisms of the soil is evident from the fact that if sterilised soil be used no such change takes place. One of the organisms of denitrification, viz. Bacterium denitrificans α, does not produce free nitrogen if asparagin be supplied to the nutrient medium (saltpetre bouillon), but instead it produces these two oxides of nitrogen. In spite of the fact that it has been known for a long time that some bacteria can effect this kind of reduction, very few positive results have been obtained, and no bacteria have hitherto been isolated which *normally* effect the reduction of nitrates and nitrites into nitrous and nitric oxides.

The third kind of reduction is that known as denitrification, as a result of which nitrates and nitrites are reduced to free nitrogen, which in consequence escapes into the atmosphere. Denitrification is caused by bacteria which doubtless utilise the oxygen which they thereby gain for purposes of respiration. The activity of a denitrifying microbe in bouillon and similar culture fluids is readily recognised by the foam on the surface of the fluid, which is caused by the escaping nitrogen. In a saltpetre-bouillon culture fluid as much as 79 per cent. of the contained nitrogen may disappear in this way. It is consequently necessary to prevent manure remaining

under conditions which are known to be favourable to the growth of denitrifying bacteria. To obtain a knowledge of these conditions it was necessary to study the organisms as pure cultures, and these were not difficult to obtain. The first two that were isolated were named respectively Bacterium denitrificans α and Bacterium denitrificans β. These were followed by Bacillus denitrificans, Bacterium dentrificans, and Bacterium Stutzeri. It is interesting to note that Bacterium denitrificans can reduce nitrates to free nitrogen only when in symbiosis with Bacillus coli communis or Bac. typhi abdominalis. Of late years several others have been described which need not be considered here. The results of these researches all tend in the same directions with regard to the physiological characteristics of the denitrifying bacteria. The most important of these is the fact that they can thrive only when an abundance of assimilable organic food and a nitrate, *e.g.* saltpetre, are presented to them together. Another important fact which has been elicited is that though they require the presence of oxygen, yet they thrive best when only a small amount of that gas is present. In the soil these conditions do not generally hold, and the danger from denitrifying bacteria lies in the congested manure heap rather than in the soil. In the latter the conditions are more favourable to the nitrifying bacteria, but in congested manure heaps the aeration is necessarily small, the amount of organic matter large, and if, through ignorance or accident, a good supply of a nitrate, *e.g.* saltpetre, is added, a great amount of nitrogen loss through denitrification will almost inevitably take place. In practice, however, it very seldom happens that all three factors are combined together, so that loss of nitrogen in this manner is not a source of great anxiety to the agriculturist.

The denitrifying organisms are widely distributed in nature, for they are found in the air, in water, and in all cultivated soils; also in the excrement of all herbivorous animals and all animals partly herbivorous and partly carnivorous. They are absent from uncultivated soils, and from the excrement of purely carnivorous animals.

Seeing that the activities of these organisms is of such great practical importance to the agriculturist and manufacturer, it is a pity that their morphological characteristics have been so much neglected. There is still considerable vagueness in the description of the various species of denitrifying bacteria—a vagueness which has probably not infrequently led to a particular organism being designated by different names.

CHAPTER XV.

FERMENTATION.

§ 1. INTRODUCTION.

THE word *fermentation* is probably derived from the Latin word *fervere*, meaning "to boil," or "to seethe." This gives us the clue to the popular conception of this term. When a lump of yeast was placed in a sugar solution, the liquid appeared to boil or seethe, without the application of heat. To the people of the pre-scientific age, yeast was just a lump of clay-like material that possessed the wonderful power of changing sugar into alcohol. Here was a process that clamoured, if not for an explanation, at least for a name. The term "fermentation," therefore, probably arose in connection with this property of yeast, the discovery of which dates into the far past; Bacchus was worshipped by the Greeks as the god of wine, and among the ancient Egyptians the knowledge of the process of making wine was regarded as a gift from Osiris himself. We do not find even an attempt at an explanation of this process until Valentinus of Erfurt, in the fifteenth century, declared that fermentation (*i.e.* yeast fermentation) was a process of purification. When sugar is fermented with yeast, the latter falls to the bottom after the process is over, leaving the upper portion of the liquid clear. Valentinus thought that the alcohol was there all the time, and that the function of the yeast was to separate it from the other constituents. It is not necessary to follow the steps which led from this crude and incorrect explanation to our present knowledge of the subject. Instead, we will deal with one or two special cases of fermentation in the light of modern research, and for this purpose we cannot take, as our first example, a better instance than this yeast fermentation.

§2. THE NATURE OF YEAST.

A pennyworth of yeast is made up of many thousands of round or oval cells, each one of which is an independent organism. Under the microscope each has the appearance of a miniature ball (Fig. 118). Within each ball are found:

1. The protoplasm or living matter.
2. A number of substances which are destined to build up fresh protoplasm or have been formed by the breaking down of the latter.

In fact all the essential activities which we associate with living organisms are all performed within the compass of each little

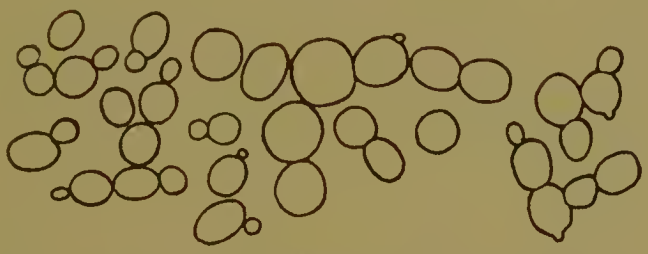


FIG. 118.—Group of cells of Saccharomyces cerevisiae. (After Hansen.)

globule. Each one, during the process of multiplication, absorbs food from the medium in which it is growing; it elaborates this food into protoplasm; it respires, as a result of which energy is liberated and waste products excreted. Finally, each is able to reproduce itself. This normally takes place by a process of **budding**. A small wart-like protuberance appears on the surface of the cell. This becomes larger and larger, until it is as large as the cell which gave birth to it (Fig. 119). This daughter-cell when mature has exactly the same structure as the mother-cell. This mode of formation of new cells is called **budding**. The daughter-cell in turn buds off a cell by the same process, whilst the mother-cell forms another daughter-cell at another point, and so the process goes on until a large number of cells are formed. Sometimes each cell, as soon as formed, is completely separated from the cell that bore it; but often the process of budding takes place more quickly than that of separation, so that bunches of cells are seen clinging together, all originally derived from one cell. The fermentation that is going on, the apparent "boiling," is

FIG. 119.—Stages in formation of new yeast-cells by process of budding.

due to the activities of these living cells—their growth, reproduction, nutrition, etc. When the nutritive matter is exhausted, or when from any other causes growth and multiplication are no longer possible, the fermentation ceases. There will obviously be a greater number of yeast cells at the end of fermentation than there was at the beginning. When a little of the yeast that is left over is placed in a fresh stock of the nutritive medium, fermentation begins once more.

§ 3. EXAMPLES OF FERMENTATION.

Yeast Fermentation. The active growth and multiplication of yeast has resulted in a transformation of the sugar into alcohol. It will at once be seen that the connection between the life of the yeast and the fermentation of the sugar is a very close one, and it would appear at first that the connection is of the same nature as that of the act of respiration to the living activities of the cells of our own bodies. But such is not the case with regard to this particular fermentation, for it is possible to kill the yeast cells without retarding the activity of the fermentation, and, on the other hand, it is possible to stop the fermentation without necessarily killing the yeast cells. The conclusion is therefore arrived at that, in this instance, though the fermentation originates from a living organism, its further activity is not bound up with, and that it does not cease with, the life of the organism which initiated it. What has happened is, that a substance has been secreted by the yeast which, though present only in small quantity itself, has the power of converting the sugar into alcohol. This substance is called a **ferment**, and the process set up by it **fermentation**. The ferment of yeast is called **zymase**. The transformation of sugar into alcohol liberates energy, which is utilised by the yeast cells to carry on the work of life.

Malt Fermentation. Our next illustration is drawn from another familiar fermentation, viz. that resulting in the conversion of starch into sugar through the agency of the ferment contained in malt. The process of malting essentially consists in strewing barley grains on the floors of the malting-house, and providing them with as much heat and moisture as is sufficient to cause germination. When the germination has reached a certain point, the maltster puts an end to the process by raising the temperature. During germination the barley secretes a substance which has the power of converting the starch contained in the barley, into sugar. This particular secretion is known as **diastase**, and germinated barley grains containing diastase

are known as **malt**. When malt is placed in water the work of conversion takes place rapidly, and a sugary solution, called **wort**, is the result. In this case the gain to the plant by the secretion of the ferment is that a digestible substance (sugar) is placed at its disposal in place of an indigestible substance, viz. starch.

Acetic Acid Fermentation. Our third illustration is introduced because in it and in similar cases we see fermentations that apparently take place without the secretion of ferments. When beer or wine is left exposed to the atmosphere, after a few days a thin skin or pellicle is found on the surface. If the liquid be examined later, it will be found that the alcohol has been changed into acetic acid, the conversion being brought about by bacteria which thrive in the beer or wine, and effect the change. The skin is made up of millions of bacteria, which correspond to the yeast and barley in the two fermentations that we have just considered. But whereas in those cases definite ferments are secreted, in this case there is no indication of such bodies, and the conclusion that must be arrived at, is, that the change which we call fermentation is effected by the organism *as a whole*, and not through the agency of a ferment secreted by it. At the same time, it is not beyond the bounds of possibility that the ferment is there, but that modern methods of dealing with ferments have not succeeded in establishing its existence.

§ 4. DEFINITION OF FERMENT AND OF FERMENTATION—PROPERTIES OF FERMENT.

We are now in a position to define what is meant by these two terms. **Fermentation** is the term applied to the decomposition of substances into simpler forms through the agency of living organisms. If there is a special secretion for this purpose, the secretion is called a **ferment**.

It must be added, however, that this definition is not universally accepted. It is claimed, for instance, that some ferments can not only decompose complicated substances, but can also build them up from simpler materials. Even if this were proved in the case of a few ferments, it would not seem advisable to extend the conception of fermentation to this length, for the logical outcome of it would be that we should have to dignify by this name any process of any kind performed through the agency of a living organism. Such an extension of the meaning of the term would serve no useful purpose, and would result in some confusion, for it would make the wall of partition between **fermentation** and **metabolism** extremely thin.

On the other hand, the term fermentation is restricted by some to those processes only which are brought about as the result of the secretion of a definite ferment by living organisms. This, however, would mean that such processes as the change of alcohol into acetic acid through the agency of the acetic-acid bacteria, the souring of milk through the agency of the lactic-acid bacteria, and other similar processes, which since the beginning of observations on this subject have always been regarded as being the most typical of fermentative processes, would be thrust out altogether. Further, the nature of the process in these cases is exactly the same as in those from which ferments have been extracted, so that there seems no reason for their separation.

Properties of Ferments. Some ferments are excreted into the surrounding medium, there to carry on their work; others, though not excreted, can easily be extracted from the living organism by glycerine or water. A third class of ferments can pass through the cell-wall only after the death of the cell, whilst a fourth kind remains inside the cell even after the death of the latter, and can be reached only by breaking up the cell-walls. No ferment has been extracted in a pure condition. An analysis of the purest form yet obtained shows that carbon, hydrogen, oxygen, and nitrogen enter into their composition, but more than this cannot be established, for the process of further extraction with the object of obtaining a purer ferment has resulted only in a weakening of its power.

A remarkable characteristic of ferments is their **selective** power. The same results achieved by them can often be produced by purely chemical means. Thus, dilute acids as well as diastase can change starch into sugar; but whilst acids can act on almost all substances, a ferment is strictly confined to one, or at most a very small number of closely allied substances.

Most ferments cease their activity at 70° C. Hence, in all fermentative industries great care is taken that this temperature is not exceeded in cultivating the cells which secrete the ferment. The presence of ferments is made known solely by their action, for they do not respond to any specific chemical test. In general they are partly precipitated by alcohol, and are also carried down when a precipitate such as calcium phosphate is produced in solutions containing the ferment. It has been asserted that they give certain colour reactions, *e.g.* with orcin and sulphuric acid, but it is more than suspected that these colour reactions are due to the impurities that are always present in ferment solutions.

As a class, ferments possess a slight degree of diffusibility, some being more diffusible than others. With regard to the amount of

ferment which is necessary to effect any particular change, it is found that a very minute quantity of some ferments is quite sufficient to effect a considerable fermentation; in fact, the weight of the fermented substance may be several hundred thousand times the weight of the ferment. Thus, a very small amount of rennet will coagulate a very large quantity of milk in the course of a few minutes. At the same time, the amount of fermentation is not altogether independent of the amount of ferment originally added, the fermentation being greater when a larger amount of the ferment is added.

In spite of the fact that ferments can act quite apart from the living cell, they do not seem to be altogether devoid of the properties we associate with living cells. They are pre-eminently susceptible to external influences; in neutral solutions they work either not at all, or in an extremely weak manner. Some absolutely require that a small quantity of acid should be present; others, a small quantity either of acid or alkali. When the solution is made more acid, up to a certain percentage, which is very small, the fermentative activity increases; but beyond this percentage decrease follows till a point is reached at which the fermentation stops altogether, and subsequent neutralisation will not cause the process to begin again. Further, the presence of certain metallic salts or of antiseptics has an injurious effect on the activity of ferments, though in this respect they possess more resistance than living cells. The influence of temperature on ferments is somewhat similar to its influence on living cells. For all living organisms there are minimum, optimum, and maximum temperatures of growth. Ferments behave in the same way. The minimum usually lies near 0° C. and the optimum between 40° C. and 60° C., whilst the maximum is never as high as 100° C. and very seldom above 70° C. If exposed to a high temperature, ferments do not regain their activity when the temperature is again lowered; but, on the other hand, their activity is only arrested, not destroyed, by exposure to low temperatures.

Ferments are sometimes described as **catalytic** agents, *i.e.* agents which induce reactions to take place without being finally altered themselves by the reactions. Many such are known to chemists. Thus, finely divided platinum acts as a catalyst in inducing the combination of sulphur dioxide and oxygen to form sulphuric acid: also, dilute acids act as catalysts in inverting cane-sugar. It is possible that when the action of inorganic catalytic agents is fully explained much light will also be thrown on the action of ferments. In the meantime, it will greatly facilitate an understanding of the subject if the student

regards ferments as catalytic agents of complex constitution, derived by secretion from a living cell.

Ferments are not universally present at all times, but are secreted only when they are required. Thus, whilst the ferment diastase is not found in barley grains, it is present in malt. Again, in many cases, perhaps in all, the organism first secretes a substance called **mother-of-ferment**, which, later, is transformed into the ferment. Other names for mother-of-ferment are **proferment** and **zymogenes**. These may be found in resting seeds and in other parts of plants where nutriment is stored. Thus **pepsinogen**, the proferment of pepsin, has been found in the resting seeds of lupin, the pepsin itself not appearing until after germination.

§ 5. CLASSIFICATION OF FERMENTS.

We may summarise what has been said above in the statement that there are two kinds of ferments.

(*A*) **A secretion of an organism**, which is able to carry on its fermentative process quite apart from the organism which produced it. Such secretions have been called **unorganised ferments, enzymes,** and **soluble ferments.**

(*B*) **The living organism itself,** working a fermentative change, only during its own processes of growth, multiplication, etc. Such organisms have been called **organised ferments.**

It is most probable that several of the fermentations at present assigned to the second class belong in reality to the first class, but that it is owing to our insufficient knowledge that we have not yet been able to extract the ferments from the cells. For instance, it was supposed that the yeast fermentation belonged to the second class until Buchner succeeded in demonstrating the presence of a ferment.

Secretions of living organisms which set up fermentation. The unorganised ferments which have been extracted may be classified as follows:

I. Ferments that change insoluble carbohydrates into soluble sugars.

Diastase (in various forms), which acts on starch and its allies, changing them into dextrins and maltose.

Cytase, which decomposes cellulose.

Inulase, ,, ,, inulin.

Seminase, ,, ,, mannose and galactose.

Pectinase, ,, ,, pectins.

II. Ferments which break up biose sugars into simpler sugars.

Invertase attacks cane-sugar, changing it into equal quantities of glucose and fructose.

Maltase changes maltose into d-glucose.

Lactase decomposes lactose or milk sugar, changing it into glucose and galactose.

Others are raffinase, trehalase, and melizitase, which decompose respectively the sugars raffinose, trehalose, and melecitose.

III. Ferments which split up **glucosides**, giving rise to glucose and various other substances. The best known are:

Emulsin, which decomposes the glucoside amygdalin into glucose, benzaldehyde, and hydrocyanic acid. It can also decompose the glucosides arbutin, salicin, and helicin.

Myosin changes potassium myronate into glucose, potassium hydrogen sulphate, and essential oil of mustard.

Others are tannase, erythrozyme, and rhamnase.

IV. The proteolytic ferments which decompose albuminous substances.

Pepsin is found chiefly in the stomachs of the higher animals. In vegetable tissues its occurrence is doubtful. It splits up albuminous substances into **peptones** and **albumoses**.

Trypsin carries the decomposition much further. The first products are probably the same as for pepsin, but the final products are **ammonia**, **amido acids**, and **di-amido acids**, which are relatively simple bodies of known constitutions. Trypsin is more widely distributed as well as more energetic than pepsin, and is found in many animals and plants.

Galactase. Found in milk, the proteids of which it hydrolyses, carrying the decomposition to the extent of the liberation of ammonia.

Papain. Found in the fruit of the papaw tree. It decomposes proteids, and in its action is intermediate between pepsin and trypsin.

V. **The fat-splitting ferment** (Lipase). Changes fats into fatty acids and glycerine. This ferment is found in all mammalian animals, the chief seat of its formation being the pancreas. It is also fairly widely distributed among the lower animals. In the vegetable kingdom it occurs in the seeds of plants which store fat as a reserve material, *e.g.* castor oil plant.

VI. **The clotting ferments.** The result of their action is the formation of a semi-gelatinous clot, which subsequently shrinks and becomes semi-fibrous.

Rennet. Found in the pancreas of many animals, in various insectivorous plants, in fruits, and other parts of plants. When it is added

to milk, the latter stiffens into a clot, and out of it a watery fluid (whey) oozes. The clot is derived from the principal constituent of milk, viz. **casein**, and is the crude cheese.

Thrombose, the fibrin ferment. Freshly drawn mammalian blood on exposure rapidly forms a clot, from which after some hours a yellow liquid (serum) exudes The clotting is due to the ferment thrombose, which is not present in normal blood, and can be first demonstrated only when coagulation takes place.

Pectase. Ferment which forms jellies from many ripe fruits. It acts on one of the constituents of the cell sap, called **pectine.**

VII. **Urease**, which sets up ammoniacal fermentation in urine. Urine on exposure changes from an acid body to an alkaline, owing to the decomposition of the contained urea into ammonium carbonate. This decomposition is due to the agency of urease, which is secreted by the organisms which drop into the urine from the atmosphere.

VIII. **Oxidases, or oxidising ferments.** These are ferments which promote the oxidation of various substances by acting as carriers of oxygen to the latter. The best known are:

Laccase, which is concerned in the production of lacquer varnish, from the sap of the lac tree of south-east Asia.

Tyrosinase. Found in several fungi and in some roots. It oxidises the **tyrosin** which is contained in these plants.

Œnoxydase, which causes the disorder in wines which is called "casse." The wine loses its characteristic colour, and a red-brown precipitate is thrown down.

IX. **Zymase**, the alcohol-producing ferment. This is the ferment secreted by yeast. It effects the decomposition of sugar into alcohol and carbon dioxide. Zymase has also been extracted from peas, from barley, and from certain fruits, *e.g.* cherry. The yeast plant can set up alcoholic fermentation in mare's milk, as is done in the preparation of the Russian beverage known as **koumiss**, and in cow's milk, as in the preparation of **kephir.** The various alcoholic fermentations will be dealt with in a later chapter.

§ 6. FERMENTATIONS FROM WHICH NO SOLUBLE FERMENTS HAVE BEEN EXTRACTED.

In this group, the fermentation in each particular case is caused either by the secretion of a ferment, the extraction of which has not as yet been accomplished, or by the organism as a whole without the

secretion of a specific ferment. We will now describe the best-known examples of such fermentations.

I. **Lactic acid fermentation.** From an industrial point of view this fermentation is of very great importance, as will be seen in the next chapter, and in point of distribution is probably more widespread than any other. Lactic acid may be produced by the fermentative decomposition of many sugars, including milk-sugar, cane-sugar, mannite, and sorbite. It is formed when plant infusions, wort and other liquids are left exposed to the atmosphere, and it has also been detected in the stomach and other parts of the body. The organisms that are responsible for these changes are numerous and widely distributed. The best known are **Bacillus acidi lactici, Bacillus lactis aerogenes, Streptococcus acidi lactici, Micrococcus acidi lactici,** and **Micrococcus lactis I.** and **II.** In the decompositions of these bacteria, lactic acid is the chief product. There are other bacteria which produce this acid as a subsidiary product, or under exceptional circumstances as a chief product. Among these are the pathogenic Bacillus coli communis, Bacillus typhosus, and Vibrio cholerae.

Although no ferment has as yet been extracted, this fermentation in some of its characteristics shows evidence of the probability that such a ferment does exist. Thus, some of the lactic-acid bacteria, when grown for a long time in a medium devoid of sugar, if now transferred into a sugar-containing medium, are found to have lost the power of changing sugar into lactic acid. But if, after losing this capacity, the bacteria are once more grown continuously in a sugar medium, the capacity of changing sugar into lactic acid is gradually regained. Again, the addition of certain metallic salts, *e.g.* corrosive sublimate or copper sulphate in very small quantities, causes an increase in the intensity of fermentation, but a decrease in the power of multiplication. This is the case in all fermentations in which the secretion of a definite ferment has been demonstrated.

II. **Acetic acid fermentation.** When an alcoholic liquid is left exposed to the atmosphere it acquires a sharp acid taste. This is due to the formation of acetic acid by the agency of various bacteria which fall into the liquid and create this fermentation. The manufacture of vinegar is an example of such a fermentation. The best known acetic acid bacteria are Bacillus aceti (Fig. 123), Bacterium Pastorianum (Fig. 124), Bacterium xylinum, and Bacterium Kützingianum (Fig. 125). The decomposition which takes place is the same as that effected by chemical oxidising agents, viz. a conversion of the alcohol into the aldehyde, and afterwards a further oxidation of the latter into acetic acid.

The acetic-acid bacteria are very strongly aerobic, and form on the surface of the liquid in which they are growing characteristic white or grey films, consisting of agglutinated masses of these bacteria. The so-called **vinegar plant** is made up of large numbers of rod-shaped individuals of Bacterium xylinum. It grows on the surface of the culture fluid, and effects the transformation of alcohol into acetic acid.

III. **Butyric acid fermentation.** Butyric acid is a common ingredient of stale milk and rancid butter, and is the chief cause of their unpleasant smell and taste. The acid is formed by the fermentation of many substances, the best known being lactic acid, glycerine, starch, mannite, and sugar.

When sugars are broken down by lactic-acid bacteria, as, for example, in the souring of milk, the resulting lactic acid is converted by butyric-acid bacteria into butyric acid, carbonic acid, and hydrogen. The best known of these organisms is one discovered by Pasteur, and called by him **Vibrion butyrique.**[1] This organism is now known as Bacillus butyricus (Pasteur), and is interesting, because it was whilst studying its life-history that Pasteur alighted on the fact that there were organisms which could live without oxygen. This bacillus is widely distributed in nature, and grows best at a temperature of 40° C. Normally it forms spores, the spore-containing individuals having the characteristic appearance shown in Fig. 43. The substances which it decomposes are lactic acid and substances like sugar, which can readily give rise to lactic acid, also tartaric, citric, and malic acids, as well as a number of other substances. In addition, it can digest cellulose, and, indeed, very probably plays an important part in the digestion of this substance inside the bodies of the higher animals.

Other butyric-acid bacteria have been isolated. One of these is **Bacillus butylicus**, which decomposes glycerine, the chief products of the decomposition being butyric and lactic acids, butyl alcohol, carbon dioxide, and hydrogen. This differs from the preceding in being a facultative, not an obligative, anaerobe. Another is **Bacillus acidi butyrici**, an anaerobe which was isolated from mixtures of sugar solution and bad cheese or rancid cream-butter. It forms spores, and, unlike the others, can liquefy gelatine. A fourth member of this group is **Bacillus ethylicus**, which can ferment thin starch paste in the presence of powdered chalk. It first of all secretes a diastatic ferment,

[1] This organism was named *Clostridium butyricum* by Prazmowski, but it has since been demonstrated that the bacteria designated by this name consisted of a number of closely allied but distinct species; that is to say, Prazmowski did not work with pure cultures.

which changes the starch into sugar, and then converts the sugar into butyric acid.

Altogether, about twenty species of butyric-acid bacteria have been described, but some of them are undoubtedly mixtures of different species, so that we cannot say at present how many distinct species are in existence.

IV. **Fermentations involving changes in the nitrogenous organic compounds of the soil.** In this group may be conveniently placed the series of changes which result from the activity of the soil bacteria, as a result of which ammonium compounds, and subsequently nitrites and nitrates, are produced. Also, in this group may be included those activities that result in the formation of oxides of nitrogen or of free nitrogen. The nature of these changes have already been described (see last chapter).

V. **Fermentations involving the transformation of sulphur compounds.** As one of the results of the activities of different kinds of bacteria which decompose organic matter, sulphuretted hydrogen is formed. The sulphur-bacteria absorb this compound, and free sulphur is formed, which is later changed by them into the sulphate. We may also include in this group the changes effected by other bacteria which change the sulphates into the sulphite form.

VI. **Propionic-, citric-, and oxalic-acid fermentations.** A bacillus has been described which transforms lactic acid into two parts of propionic and one part of acetic acid, carbon dioxide and water being formed at the same time. Certain fungi also are known which bring about the formation of citric acid as a chief product. Two of these, known as Citromyces Pfefferianus and Citromyces glaber respectively, belong to the mould fungi. They produce citric acid from glucose, and under certain circumstances the amount of acid formed may be more than 50 per cent. of the glucose employed. Other fungi, notably Penicillium and Sclerotinia, are known to change sugar into oxalic acid, and this power is possessed also by certain yeasts, *e.g.* Saccharomyces Hansenii, by the aid of which large quantities of calcium oxalate may be formed.

CHAPTER XVI.

INDUSTRIAL APPLICATIONS OF FERMENTATIVE PROCESSES.

§ 1. INTRODUCTION.

In a work of this nature the minuter details in the fermentation of any particular industry cannot be entered into. It will be sufficient if we indicate the general principles which are followed. In all such industries, what is aimed at is the effecting of a particular change in the substance through the instrumentality of a particular living organism. But as the atmosphere is charged with different kinds of micro-organisms, it is often difficult to keep these out. If they gain entrance, they may set up fermentations which are altogether undesirable and spoil everything. Hence to ensure the predominance of the organism, which sets up the desired fermentation, recourse may be had to two expedients:

1. To cultivate it under conditions of temperature, moisture, etc., which are known to be best suited to its growth.
2. To give it a start by weakening its competitors.

If the organism has been isolated, the conditions most favourable to its growth are easily ascertained, and in some of the older fermentative industries the practical experience of centuries has resulted in a very accurate knowledge of the most favourable conditions. The weakening of the competitors is sometimes ensured by the application of heat to the substance to be fermented before the introduction of the desirable organism. In other cases a substance exercising a deleterious effect, which is less injurious to the desired organism than to its competitors, may be introduced. As the organisms are different for each industry, it follows that the conditions insuring the best results will be different. What will remain the same, however, will be the principles governing

the ascertainment of these favourable conditions. The production of alcohol by yeast-fermentation has reached such a high pitch of excellence that, when conducted on the most approved methods, failure is almost impossible, in spite of the fact that wort is a suitable medium for the growth of many bacteria and other small organisms. A list of fermentative industries could be made, graded according to the scientific knowledge of the processes involved. The bottom of this list would include those fermentative processes which are entirely left to chance, and in which even the organisms responsible for the fermentative changes are unknown. With regard to the majority of such processes the sum total of our knowledge is extremely small, though here and there small additions are being constantly made.

§ 2. THE PRODUCTION OF ALCOHOL.

As already mentioned, this is the oldest fermentative industry of which we have any knowledge. We learn from Herodotus that the ancient Egyptians made wine from barley, and we are told by Pliny that all the nations of Western Europe made beer. In some form or other alcoholic beverages are partaken of, by almost every people on the face of the earth.

The methods adopted in such an excellent brewery as the Carlsberg Brewery in Copenhagen represent the highest pitch of excellence that any fermentative industry has yet achieved. In principle the production of alcohol is simple. Into a sugary solution is introduced one or other of the Yeasts. This is allowed to grow and multiply, the result being the transformation of the sugar into alcohol.

(*a*) **Beer.** In making beer, the brewer does not start directly from sugar, but from starch, which can readily be converted into sugar. We must therefore distinguish two phases:

(i) The production of sugar from starch.
(ii) ,, alcohol from sugar.

We have already explained how barley grains are converted into malt, and have noted that the latter contains essentially starch and the ferment diastase, which can change this starch into sugar. In the malt condition, however, diastase is ineffective, owing to the absence of moisture. The process of brewing may be divided into six stages:

1. **Grinding.** The malt is bruised or crushed, and left in a heap for a few days to allow it to mellow. This enables the diastase to be more easily abstracted.

2. **Mashing.** The bruised or crushed malt is thrown into the mash tun, and water is added at a temperature of from 158° F. to 172° F. After maceration for three or four hours, assisted during the first half-hour by constant stirring, the liquid portion is strained off through finely perforated plates in the bottom of the mash-tun and pumped into the copper. The mashing has converted all the starch in the malt into sugar, through the agency of the ferment diastase. The temperature must not be below 140° F., otherwise the diastase will not work, and must not be above 185° F., for this temperature will destroy the diastase altogether. A medium temperature of about 165° F. is found to be the most suitable. The sugary liquid that is now obtained is called *wort.*

3. **Boiling.** When the wort is all collected into the copper, hops are added and the whole is boiled for about three hours. The boiling coagulates and precipitates the excess of albumen that is present, and also extracts the aromatic oil and the more pronounced of the bitter substances of the hops. This process serves several purposes. The boiling kills all the minute-organisms that are present, and by removing the albumen prevents putrefactive fermentations setting in later, whilst the hops not only give a characteristic flavour to the beer, but by imparting its bitterness prevent too rapid fermentation when yeast is added.

4. **Cooling.** After the boiling is finished, the wort is cooled as quickly as possible by exposing it to a current of air in large shallow vessels, or running it over refrigerating pipes. Wort is capable of serving as a nutrient medium to various kinds of bacteria and moulds which are always in the immediate vicinity; most of these, if they get a chance to multiply in the wort, would produce acid decomposition-products, and thus would render it unfit for the next stage, because yeast cannot multiply in an acid medium. At a low temperature, however, these organisms, even if they find their way to the wort, cannot multiply.

5. **Fermenting with Yeast.** The wort is next run into fermenting vats at a low temperature and yeast added. We have already explained how yeast, by multiplying in the wort, changes the sugar into alcohol. The fermentation is allowed to proceed for about 48 hours, when the yeast is skimmed off if it collects at the top, or is run out through holes in the bottom of the tun if it forms a sediment at the bottom. Yeasts can be roughly divided into two groups—the *Top-* and the *Bottom-Fermentation Yeasts*, so called because, when cultivated in nutrient media containing a fermentable sugar, the former always form a froth

at the top which is thick with yeast, whereas the latter either form a thin layer only of yeast at the top, or else none at all.

6. **Cleansing.** Finally, the action of yeast is checked by a process of cleansing which we need not enter into. The finished beer varies in specific gravity from 1·002° to 1·030°, and contains from 4 to 24 per cent. of proof spirit. There will be more yeast at the close of fermentation than at the beginning. A portion is kept aside and used for the next fermentation, the remainder being sold or otherwise disposed of.

As in former times no special precautions were taken with the fermentation, it is not surprising that occasionally everything went wrong because other organisms had got into the wort and got mixed up with the yeast. Some of these intruders produced injurious products which resulted in heavy financial losses. What was called yeast was often a mixture of several organisms, and the brewer never knew when one of the undesirable constituents was going to predominate in the wort and spoil the fermentation. When Pasteur's doctrine that bacteria were responsible for the diseases of fermented liquids became accepted, a cry arose for the purification of brewer's yeast, as it was thought that bacteria were responsible when a yeast fermentation went wrong. It was, however, subsequently shown by the work of E. Chr. Hansen that some of the worst diseases of fermented liquids were due to "foreign" or "wild" yeasts that had gained access to the wort. A wide-reaching reform was effected when Hansen separated the culture yeasts from the wild yeasts, and eliminated the latter from the breweries of Copenhagen. His researches further resulted in the separation of the culture yeasts into several species and races, the best being adapted for fermentation. His new system of employing pure cultures spread quickly into different countries, and was adopted not only by the breweries, but by the spirit, pressed-yeast, and wine industries. It is regrettable that Great Britain has not yet taken full advantage of these discoveries. For a detailed description of the species and races of yeasts, the student may refer to Klöcker's *Fermentation Organisms*, in which much useful information on this subject may be obtained. A good example of a "wild yeast" is afforded by Saccharomyces Pastorianus I., discovered by Hansen in the air at the Alt-Carlsberg

FIG. 120.—Saccharomyces Pastorianus I. (After Hansen.)

Brewery, Copenhagen. This species had crept into the stock yeast of this brewery, imparting to the beer a disagreeable bitter flavour and a smoky smell (Fig. 120). The cells have a strong tendency, as will be seen by the diagram, to run into the sausage rather than the normal globular or oval shape. As an example of a culture yeast, we may take Saccharomyces cerevisiae I., isolated from a top-fermentation brewery in Edinburgh (Fig. 118).

Not only "wild" yeasts, but also bacteria, compete with the culture yeasts for the mastery of the wort. There are, however, only a few species which can do harm to any appreciable extent, because the bitterness of the wort inhibits the growth of most bacteria, whilst the introduction of the pure culture system renders more certain the success of the culture yeast. Bacteria cause mucilage formation, decolorisation, turbidity, acid formation, and disagreeable smells and tastes.

A common complaint in beer is **ropiness**. When thus affected, the beer becomes thick, mucilaginous, and capable of being drawn out into threads. Two of the bacteria causing this disease have been isolated from Belgian ropy beers, and named respectively Bacillus viscosus I. and Bacillus viscosus II. These bacteria form rods about 0·8 μ broad and 1·6-2·4 μ long. They differ in that beer affected with the first shows yellowish-white viscous patches on the surface, which are wanting in beers infected by the second. A third, called Bacillus viscosus III., has been isolated from British ropy beers. The ropiness is due to a change in the cell-wall of the bacteria, which becomes mucilaginous, and not to any compound formed in the beer. Finally, ropiness may be caused by the ravages of a member of the Mould Fungi, called Dematium pullulans. This fungus has a branched, thread-like structure (Fig. 121*a*), and also forms a number of yeast-like cells (Fig. 121*b*). Another defect in beer is the production of **turbidity**, which may be caused in several ways. It may be due to the precipitation of albuminoids (*gluten turbidity*), or to the presence of unsaccharified starch (*starch turbidity*), or to a high content of yeast-cells (*yeast*

FIG. 121.—Dematium pullulans. (*a*) Tubular cells, forming conidia; (*b*) cells dividing up to form short swollen cells. (After Zopf.)

turbidity), or, finally, to a strong infection of bacteria (*bacterial turbidity*). In the last-named case turbidity may arise either as a secondary result of ropiness-producing organisms, or may be due to the infection of organisms which do not at the same time produce ropiness. The organisms causing the last-named disease are members of the sarcina group and the disease is known as *sarcina-turbidity*. It has been shown that these sarcinae, however, may be present in beer in very large numbers without appreciably affecting the quality, and that the malady arises only if certain species are present, and then only under certain conditions.

Bitterness in beer is caused by the undue development of the "wild" yeast Saccharomyces Pastorianus I. According to Hansen, not only the taste and odour, but also the stability of beer is affected by the presence of this organism in such small quantities as one-fifth of the pitching yeast.

Other "wild" yeasts of the same class are Saccharomyces Pastorianus II. and III. These also induce the development of volatile substances which impart to the beer a stronger taste and smell than are produced by the culture yeasts. No. III., if present in an appreciable quantity, also induces turbidity. Although a source of danger, it is claimed that the addition of a small quantity of No. III. to the stock yeast is beneficial when the beer is apt to be opalescent.

The **turning** of beer is caused by a bacterial species, known as Saccharobacillus Pastorianus. The beer loses its brilliancy, has a disagreeable smell and taste, and forms a sediment. This species develops in beer only when the latter contains a small amount of hop extract. Its development in beer is prevented when more than 7 per cent. alcohol is present.

Some members of the acetic-acid bacteria may cause much damage, especially in top-fermentation breweries, where the conditions for their development are more favourable than in bottom-fermentation breweries. By producing acetic acid they cause an unpleasant sourness, which greatly detracts from the value of the beer thus affected. The best known are Bacterium aceti, Bact. Pastorianum, and Bact. Kützingianum. As these bacteria are strongly aerobic, beer infected by one or more of them does not become sour if the bottles are well closed and well filled.

Finally, mention must be made of the ravages that may be caused by the accidental entrance of lactic-acid or butyric-acid bacteria into the mashing- or fermenting-tuns. The butyric-acid bacteria are

particularly obnoxious, if they multiply, for a small quantity of butyric acid can go a long way towards spoiling the whole brewing.

(*b*) **Whiskey.** The preparation of whiskey does not differ in essentials from the process just described for the production of beer, except that, as a much higher percentage of alcohol is desired, distillation is in addition resorted to, in order to achieve this end. There are two varieties, viz., *malt whiskey* and *grain whiskey*. The former, which is of finer quality, is made principally from malted barley, or, more rarely, from rye. The latter is cheaper, but stronger, and is made from various substances, as sugar, molasses, potatoes, but principally from unmalted grain, as Indian corn, barley, oats, etc., dried and ground up. The principal ingredient in all these substances is either starch or sugar. The fermentation is essentially the same, and as a matter of fact most of the distilleries in this country get their yeast either directly or indirectly from the breweries. The fermentation, however, is of a more difficult nature, because the liquid to be fermented, the "sweet goods," as it is called, instead of being self-clarifying, like wort, and thus easily separated from the yeast, is usually a thick mash in which the yeast cannot settle down. Instead, therefore, of directly adding the yeast to the "sweet goods" contained in the principal mash-tun, a preliminary and important fermentation is effected, which is very instructive as illustrating the manner in which a desired fermentation can be accomplished under difficulties. The distiller removes a certain small amount of "sweet goods" from the principal mash-tun, and adds to this crushed green malt which has been mixed with water, and gradually warmed to 67°–70° C. The whole mixture is next allowed to sweeten for about two hours at 70° C. Sweetening will take place because the diastase in the malt will change the starch into sugar. Now, a sugary solution of this nature is an unusually good nutrient medium for all kinds of bacteria, and the ones that are most to be feared are the species belonging to the butyric-acid bacteria, which unfortunately are almost always present. If these multiply to any appreciable extent, the whole fermentation will be destroyed. To remove all risk of this happening, the distiller acts as follows: He first introduces lactic-acid bacteria to a small portion of the "sweet goods." Now it is known that these organisms thrive best between 47° C. and 52° C., whereas the butyric-acid bacteria prefer a temperature near 40° C. He therefore keeps the temperature of the mixture at about 50° C., with the result that the acidity which develops is due to lactic acid. Then, when the necessary degree of acidity has been attained, sufficient heat is applied to cause the death of the lactic-acid bacteria.

Next, yeast is added and allowed to grow and multiply for about 14–16 hours. This gives the distiller a slightly acid liquid, devoid of injurious organisms and containing a healthy culture of yeast. This preparation is added to the mash-tun containing the bulk of the "sweet goods," when brisk fermentation takes place; the yeast rapidly multiplies, and in doing so uses up the sugar and forms alcohol.

When fermentation is completed, the product is distilled several times, the distillates becoming progressively stronger in alcohol because this substance vaporises at a lower temperature than the other constituents contained in the fermented mass.

The flavour of whiskey does not depend on the alcohol, but rather on small quantities of other volatile substances that come over with the alcohol in the distillation. The flavour of some brands of Scotch whiskey is due to the use of peat fires in the preparation of malt.

(*c*) **Wine.** Wine is prepared by the fermentation of grape juice. The expressed juice of the grape is known as *must*, and contains from 15 to 33 per cent. of the sugar *glucose*. The old-fashioned method of preparing wine consisted in leaving the fermentation to chance. *Must* usually contains one or more individuals belonging to one of the yeasts, and when left alone multiplication takes place, a portion of the glucose being used up and alcohol produced. In many places this method is still employed, and there can be no doubt that in wine-growing districts the chances are greatly in favour of the right fermentation being developed, because in such places yeasts are present in the soil in large numbers, and also because *must* is a better nutritive medium for yeasts than for other micro-organisms. At the same time there will always be an element of danger, and the modern method of using pure cultures reduces the risks of a wrong fermentation to a minimum. Thus a yeast known as Saccharomyces ellipsoideus I. was found by Hansen on the surface of ripe grapes in the Vosges district. There can be little doubt that this species is mainly responsible for the wine-fermentation of this neighbourhood. Its general appearance is seen in Fig. 122, the distinguishing feature being the shape of the cells, some being ellipsoidal, others being more sausage shaped. As this species was isolated and

Fig. 122.—Saccharomyces ellipsoideus I. (Hansen.)

investigated as a pure culture, the conditions determining its optimum growth became known. It is therefore obvious that if *must* be inoculated with a pure culture of this species, and allowed to ferment under conditions most favourable to its growth, the chances of a good result are much greater than when the fermentation is left to chance.

Saccharomyces ellipsoideus I. is only one of many yeasts concerned in wine-fermentation. In experimental stations for wine culture numerous species related to Saccharomyces ellipsoideus I. have been isolated. Among the best known is "Johannisberg II.," which has the peculiarity that when cultivated on gypsum blocks (the recognised method for the production of spores in yeast cells) as many as 99–100 per cent. of the individuals develop spores.

The following table gives the percentage of alcohol in the best-known wines:

	Per Cent. by Weight.		Per Cent. by Weight.
Port (average)	16·20	Hock	9·60
Sherry	15·37	,, (Rudesheimer)	8·40
Madeira (strong)	16·90	Claret	9·78
Marsala	14·60	Gooseberry	9·50
Sauterne	11·40	Orange	9·00
Burgundy (average)	11·20	Elderberry	7·40
Champagne	10·00		

The diseases which wines are liable to contract, arise as a result of fermentations set up by certain bacteria which have gained entrance

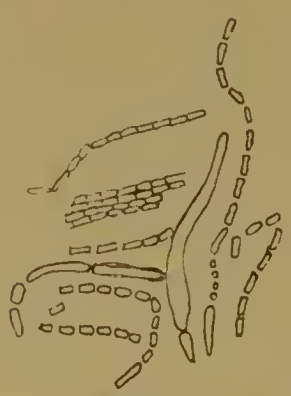


Fig. 123.—Bacterium aceti. (After Hansen.)

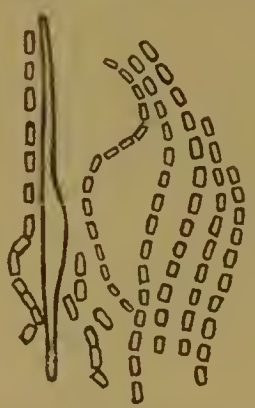


Fig. 124.—Bacterium Pastorianum. (After Hansen.)

into the wines. These bacteria produce obnoxious substances, which render the wine highly unpalatable. The number of harmful bacteria, however, is very small, because for most bacteria wine as a nutrient

medium is too acid. The commonest malady is the souring of wine, owing to the partial conversion of alcohol into acetic acid by acetic acid bacteria. This result can easily be brought about by leaving wine exposed to the air for a few days. A tough mucinous skin, composed of millions of bacteria very closely packed together, will form on the surface of the wine. These bacteria are always present in the atmosphere, so that when wine is left exposed it is not long before one of them drops in and starts multiplying, producing in a short time millions of its kind. Three of these species have been very accurately investigated, viz. Bacterium aceti (Fig. 123), Bact. Pastorianum (Fig. 124), and Bact. Kützingianum (Fig. 125).

Fig. 125.—Bacterium Kützingianum. (After Hansen.)

Ropiness is as common a phenomenon in diseased wine as in beer. Kramer has isolated from ropy wines a bacillus with which he was able to induce the same disease in sound white wine.

The disease known as "**turning**" of wine results in the assumption of a brown colour by red wines, whilst white wines become turbid and discoloured, and also often assume a dark colour. Two species belonging to the genus Micrococcus, which have these effects on wine, have been isolated.

Finally, **lactic acidification** ("Zickendwerden") may develop in wine. This is not an infrequent malady, and is caused by the development of lactic acid, as a result of infection by *Bacillus acidi lactici*. This species consists of rods which are 1·0–1·7 μ long and 0·3–0·4 μ broad; they are frequently connected in pairs, rarely in four-membered chains. Wines that in some way have lost their natural acidity are particularly liable to this disease. Thus in 1882 and 1883, the vineyards in the lowlands of Etsch (South Tyrol) were flooded, the grapes becoming incrusted with the carbonates of lime and magnesia. A result of this was that a portion of the acid in the grapes was neutralised in the process of crushing, with the further result that for a time lactic acidity became a general complaint in the neighbourhood.

(*d*) **Ginger Beer.** This popular beverage is produced in the following manner: A soda-water bottle is filled three parts full of a sugary solution, and into it is placed a lump of ginger and a few lumps of the *ginger beer plant*. In from 24–28 hours the liquid becomes turbid and bubbles of gas arise. The lumps composing the ginger beer plant are composed of several organisms, two of which cause the fermentation in question. One is a yeast (Saccharomyces pyriformis), and the other

one of the bacteria (Bacterium vermiforme) (Fig. 126). The latter has gelatinous walls, and is sometimes composed of filaments, sometimes of very short rods, and in this jelly-like mass the yeast cells are embedded. Fermentation is set up by the combined activity of these two organisms. As explained in a previous chapter, when two organisms live thus amicably together, they are said to be in a state of **symbiosis**. The turbidity which is noticed during fermentation is due to the budding off into the liquid of large numbers of yeast cells from the "plant," which then multiply and give off bubbles of the gas carbon dioxide. After a time the liquid becomes so viscous that the gas bubbles escape with difficulty from the liquid, the viscosity being due not so much to the presence of yeast-cells as to innumerable bits of gelatinous material derived from the bacterium. At this stage, also, myriads of rod-shaped individuals belonging to the bacterial partner are found in the fermenting mass. At the close of fermentation, the solution contains alcohol, acetic acid, and an incompletely known acid resembling lactic acid. The amount of proof-spirit present in ginger beer varies from 1 to 5 per cent.

FIG. 126.—Section through "Ginger-beer plant." Oval cells = Saccharomyces pyriformis, rod cells = Bacterium vermiforme.

(*c*) **Koumiss and Kephir.** These beverages are produced by the fermentation of milk-sugar or lactose, and are prepared in Russia and various parts of Central Asia.

Koumiss is a preparation obtained by fermentation of the milk yielded by mares. The milk is placed in small casks, which then receive lumps of old koumiss used in previous fermentations. A lump of koumiss consists of a mixture of several organisms, intimately blended together, as in the ginger-beer plant. One of these organisms is a yeast, whilst others are lactic-acid bacteria. These feed on the milk-sugar, producing alcohol, carbon dioxide, and lactic acid. When the fermentation is nearly completed, the liquid is placed in bottles, the latter being then fitted with tight-fitting, securely-wired corks. The fermentation is finished inside the bottles, and, as carbon dioxide continues to be given off, an effervescent beverage is obtained. Mare's milk contains about 5·5 per cent. of sugar, whilst in koumiss the percentage is reduced to 1·3. On the other hand, koumiss contains 1·6 per cent. of alcohol, and nearly 1 per cent. of both lactic acid and carbon dioxide. There is reason to believe that other changes also

take place; for example, that the casein is changed to peptone and acid albumen.

Kephir is prepared in the Caucasus from cow's milk. The kephir granules are semi-translucent masses of a gelatinous consistency (Fig. 127). These masses consist of bacteria and a yeast in symbiotic association. The gelatinous material is yielded by the bacteria, and in this the yeast cells are embedded. In the preparation of the beverage the milk is raised to a temperature of 18°–19° C., the kephir being then added. Fermentation is allowed to proceed, under constant agitation of the liquid, for about 24 hours, after which the liquid is bottled and the fermentation completed in the bottles. It results in a reduction of the percentage of the milk-sugar, the fatty matters, and the proteids, whilst it creates about 1 per cent. of alcohol and a slightly larger amount of lactic acid. There are obviously two fermentations taking place side by side, the yeast changing the milk-sugar into alcohol, and the bacteria changing the milk-sugar into lactic acid. The reduction of the fatty matters and the proteids may be due to still other fermentations, or may be also used up in the two fermentations just mentioned.

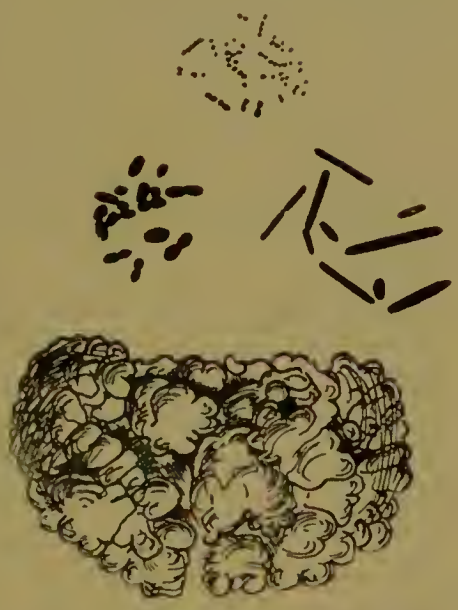


FIG. 127.—Kephir (natural size) and the species of bacteria of which it is composed. (After Freudenreich.)

§ 3. THE PREPARATION OF VINEGAR.

We have already mentioned the salient facts connected with acetic-acid fermentation. In the manufacture of vinegar, which is practically acetic acid, two methods are in general use.

(*a*) In the first method, wine is the raw material. A number of oaken casks are arranged in rows. In each is placed a small quantity (half litre) of vinegar and four times that quantity of wine. The vinegar is derived from a previous fermentation, and is added because it contains acetic-acid bacteria. The room in which the casks are

placed is maintained at a temperature best suited for the growth of these bacteria, viz. 18°–22° C., and the casks are kept closed, except for a small vent at the top, for the admission of air. The closure of the casks keeps off extraneous organisms that would otherwise drop in from the atmosphere and develop opposition fermentations; but the vent is also necessary because the acetic-acid bacteria are very strongly aerobic. At the end of every week some more wine is added to each cask, until finally each contains about forty gallons. By this time the acetic-acid bacteria have done their work, and the wine has been changed into vinegar. When once started a certain portion of the vinegar is periodically withdrawn, and its place taken by wine. A cask can be used in this way for six to eight years without interruption, but at the end of that time it has to be cleaned out to get rid of the impurities which have accumulated at the bottom of the cask.

(*b*) The other method is called the "quick vinegar method." The raw material in this case is spirit. Spirit diluted with vinegar, technically termed "goods," is run over shavings of beechwood or birchwood contained in vats which are closed except for a few vents. The acetic-acid bacteria are present in the vinegar constituent of the "goods." They settle on the shavings and ferment the alcohol, as it slowly trickles over the shavings, into acetic acid.

The number of known acetic-acid bacteria is comparatively numerous, but very few have been accurately investigated. The best known are Bacterium aceti, Bacterium Pastorianum, Bacterium Kützingianum.

Bacterium Aceti (Kützing) Hansen is frequently present in the dust of the air, and occasionally in water. It grows well in "double" beer and in wort. In the former, when cultivated at 34° C., it forms after 24 hours a smooth gelatinous film. The individuals of the film are usually hour-glass shaped, and arranged in chains (Fig. 123). At 40° C. long thin threads are formed. On wort gelatine grey, waxy, round colonies with sharp contours are formed. The colonies consist of small free rod-bacteria. The maximum temperature of growth in "double" beer is about 42° C., the minimum 4°–5° C. When a film is formed the mucilage binding the individuals together is not stained by a solution of iodine.

Bacterium Pastorianum Hansen. This species forms in "double" beer at 34° C. a dry wrinkled film after 24 hours. In general appearance the individuals are somewhat larger and thicker than those of Bacterium aceti when growing in this medium (Fig. 124). The threads which are formed when this organism is cultivated at 40° C. are similarly thicker than those of Bacterium aceti at the same

temperature. This species is further easily distinguished from the fact that the mucilage is stained blue by a solution of iodine in potassium iodide.

Bacterium Kützingianum Hansen. This species, like Bacterium Pastorianum, forms a dry film on the surface of "doppel" beer, but as this film rises high above the liquid along the sides of the flask, the species is readily distinguished from the other acetic-acid bacteria. The film consists of small rod-bacteria (Fig. 125), and only rarely forms threads. As in Bacterium Pastorianum, the mucilage of the film is stained by a solution of iodine in potassium iodide. Other bacteria capable of forming vinegar from alcohol are Bacterium xylinum, Bacterium vermiforme, Bacterium acetigenum, Termobacterium aceti, and Termobacterium luteseens. The exact relation of these species to the production of vinegar has not been extensively studied. Beijerinck mentions Bacterium aceti as the organism concerned in the quick-vinegar manufacture, but it seems to be still doubtful whether this species is identical with the Bacterium aceti which we have described above. Bacterium xylinum and Bacterium acetigenum are known to occur in the acetifiers of the quick-vinegar process.

CHAPTER XVII.

INDUSTRIAL APPLICATIONS OF FERMENTATIVE PROCESSES (*Continued*).

§ 1. BUTTER.

As is well known, butter is made by the churning of cream. A few countries, *e.g.* China and Japan, prefer **sweet-cream butter**, *i.e.* butter made from fresh cream which has not lost its sweet taste; but most other nations prefer **sour-cream butter**, *i.e.* butter made from cream which has been set aside and allowed to become sour. The process by which cream becomes sour is called **ripening**, and it is in the manipulation of the ripening that dairy bacteriologists have within the last few years achieved such excellent results. As cream is an ideal food for bacteria, it contains many thousands of bacteria per cubic centimetre, and when set aside for a short time these bacteria increase enormously, until, when ready for churning, the average number is about 300 millions per cubic centimetre. Now the sourness is caused by these bacteria, and among them those producing lactic acid are the most prominent. This must necessarily be the case, for some of the lactic-acid bacteria are extremely common in nature, and thrive better in cream than other bacteria do. There are over a hundred known species which can produce lactic acid in milk, and of these the best known are Bacillus acidi lactici, Streptococcus acidi lactici, Micrococcus acidi lactici, and Micrococcus lactis. Recent work, however, tends to show that the souring of milk is almost always accomplished by one or two species, notably Bacillus acidi lactici, the reason being that this organism thrives uncommonly well in cream, and being also so plentiful in nature, very few creams escape infection by it. This suits the purposes

of the butter-maker, because the presence of lactic acid in the cream helps him in four ways:

1. It makes the churning easier and more complete.
2. The butter keeps better.
3. The yield of butter is greater.
4. The flavour and aroma of butter are improved.

But as cream is a splendid nutrient medium for all sorts of bacteria, it will be seen that it must occasionally happen that undesirable bacteria gain the upper hand, as a result of which obnoxious substances will be formed, rendering the milk unpalatable, if not poisonous.

The modern butter-maker leaves nothing to chance. He regulates the souring in such a way that the risk of an undesirable fermentation is reduced to a minimum. This regulation is called **artificial souring**, and may be accomplished by one of the following ways:

I. **The Natural Starter Method.** This consists in adding to the ripening cream a small portion of cream from a previous favourable ripening. If the ripening has been favourable on a previous occasion, it follows that the predominant bacteria in that cream were good. By setting aside, therefore, a portion of this cream and adding it to the next ripening, a start is given to the same bacteria, and so the chances of another favourable ripening are increased. This "natural starter," as it is called, may be cream taken from a very good dairy or from the herd producing the best quality of cream. The method, however, has one defect in not being always reliable, because, as the bacterial content of the "starter" is not known and not regulated, there is always the possibility of the presence in it of harmful bacteria which are liable to drop in at any moment. Then if these multiplied, at the next ripening the whole of the next batch of butter would be rendered valueless.

The second and third methods consist in the employment of **pure culture starters.** The starter is a pure culture of an organism which is known to produce favourable changes in the cream. In debating the choice of a starter, there are two points which have to be considered. The butter-maker may elect to make sure that the souring takes place along the right lines (second method), or he may leave the souring partially to chance and employ a pure culture which ensures a splendid aroma in the butter if the souring has been successful (third method).

II. **Pure Culture Method regulating the Souring of Cream.** The starter is prepared as follows: A small portion of cream is warmed up to 60° C., then immediately recooled as quickly as possible. This kills or weakens the bacteria already present in it. Then a pure culture of the organism which is known to sour milk along the right lines is

added, after which the cream is kept for 24 hours at about 15° C. This secures the multiplication of the inoculated organism, and in 24 hours the starter, as the cream is now called, contains many millions of the inoculated bacteria, and is ready to be added to the bulk of the cream. The latter is prepared for the inception of the starter by having been previously cooled to a low temperature to weaken the bacteria contained in it. Just before the inception of the starter, the bulk of the cream is quickly heated up to 15°–20° C. After adding the starter, fermentation is allowed to proceed at 15°–20° C. till the following day, when the cream is ready for churning.

Several modifications of this method are in use, but in principle they are all the same. They differ only in the methods employed for the weakening of the undesirable bacteria before the introduction of the pure culture.

Centres like the Kilmarnock Dairy School distribute starters to various parts of the country in the form of a dry powder, containing the microbe which it is desired to introduce into the cream.

III. **Pure Culture Method regulating the Aroma of Butter.** The commercial value of butter chiefly depends on its aroma. In this method the souring is left more or less to chance, but care is taken to introduce into the ripening cream an organism which is known to act on the albuminoid constituents of cream, producing a substance which gives the butter a fine aroma. One of these organisms has been isolated by Conn and is known as **Bacillus No. 41**. The method of ripening when this bacillus is employed is as follows: Six quarts of cream are pasteurised (by heating at 155° F.), and then cooled. A pellet containing Bacillus No. 41 is thrown in, the cream being then set aside in a warm place (70° F.). After being allowed to ripen for two days, the cream, containing now many millions of the progeny of Bacillus No. 41, is added to 25 gallons of ordinary cream, which in its turn is allowed to ripen in the same way. This ripened cream is used as a starter to the large cream vats in the proportion of one gallon of starter to 25 gallons of unripened cream. The essential point to note in connection with this method is, that the bulk of the cream is not pasteurised or treated in any way so as to weaken the bacteria contained in it. As the starter does not contain a lactic-acid producing microbe, the souring must be left to the bacteria contained in the main bulk of the cream. This method naturally increases the risk of a bad ripening, but seems to work well in practice.

§ 2. DEFECTS IN BUTTER.

Whilst butter-making by any one of these three methods will very seldom be attended by serious mishaps, the close dependence of ripening on the fermentation of bacteria and the ever-changing nature of the bacterial contents in any prescribed space render butter-making a risky process to those who do not take any special precautions. It may and does sometimes happen that ripening does not proceed normally, unpleasant smells being given off, and the flavour being offensive. During the time that elapses between the milking and the churning, the cream is exposed on all sides to the attacks of many species of bacteria, and the vicinity of the byre is peculiarly rich in kinds that thrive excellently in milk and at the same time produce in it very undesirable changes. A familiar instance is the *turnip flavour* that butter sometimes possesses. The taste is somewhat sweet, and recalls that of turnips. One of the organisms responsible for this malady is *Bacillus foetidus lactis*, the individuals of which are rod-shaped and very small, usually about 0·9–1·5 μ in length, and 0·4–0·6 μ in breadth. They are normally motile and do not form spores. Other bacteria are known to be able to produce this turnip flavour, but up to the present they have not been accurately described. Artificial souring and absolute cleanliness are obviously the best means of preventing the predominance of this bacillus in the ripening cream. Almost all the other defects of butter can be traced in a similar way to the presence of malignant bacteria. Thus *bitter butter* is due to fermentative changes in the cream or subsequently in the butter, due to such organisms as Bac. fluorescens liquefaciens, Oidium lactis, and Cladosporium butyri. Again "*oily*" butter, in which the taste recalls that of mineral oil, is apt to be produced in dairies containing imperfect appliances for keeping the cream and butter at a low temperature. Although the organism causing this change has not been isolated, the change is very probably due to some microbe, for artificial souring is an efficient protection against this malady. Finally, a *fishy* or *train-oil flavour* is an evil that butter-makers have to fight against. The cause of it has not yet been discovered, but we may safely conclude that in this case also bacterial action is responsible for the malady.

§ 3. CHEESE.

Cheese is prepared by separating **caseinogen** from the other constituents of milk by the use of the ferment **rennet**. Rennet causes the

milk to stiffen to a jelly-like consistency. After standing a while this jelly contracts slightly and a watery liquid—the **whey**—oozes out of it. Then, still later, firm clots called **curds** are found floating in the whey. These clots, which are the crude cheese, are massed together, pressed, and then set aside for several weeks, sometimes for months, to undergo a process of ripening. The taste of freshly-made cheese is not pleasant, and its value depends on the changes that take place during the ripening, of which, unfortunately, we know as yet very little. That the ripening is due to bacteria is shown by the following facts:

1. It takes place best at temperatures which are most favourable for the growth of bacteria.
2. Cheese prepared from sterilised milk will not ripen; it possesses the same taste for months after being made.
3. Bacteria undoubtedly grow and multiply in cheese during ripening.
4. The substances formed during ripening resemble in some cases the decomposition products of bacterial action.

It is therefore not surprising that sometimes, when apparently the ripening has proceeded in a normal manner, it is found that a cheese has become abnormal and worthless. The bacteria present in cream are never exactly the same in any two samples, and sometimes disease-bacteria gain entrance into the cream and subsequently into the cheese. These during ripening multiply slowly, producing substances which render the cheese worthless. The ripening of cheese is a slow process, because the consistency is very dense and there is very little moisture present, so that rapid multiplication of the contained bacteria is not possible. Further, these bacteria are very probably aerobic, so that the lack of a sufficient quantity of oxygen is probably another reason for the absence of rapid multiplication. Of late years much attention has been bestowed on the bacteria that are found in ripening cheese. These are found to be of four kinds:

1. Lactic-acid bacteria.
2. Casein-digesting bacteria.
3. Gas-producing bacteria.
4. Extraneous bacteria accidentally present.

There is some evidence to show that in most cases ripening is accomplished by several of the lactic-acid and the casein-digesting bacteria and not by any one organism. It is probable that we shall, before long, be able to estimate the exact rôle played by each of the

organisms, and so be able to control the ripening, for we shall then be able to introduce starters into the cream or curds and take steps to ensure the predominance of the right bacteria.

As a matter of fact, steps in this direction have been taken, as Lloyd states that in the case of Cheddar cheese, *Bacillus acidi lactici* alone is necessary, the other bacteria tending more or less to hinder the process. In preparing this cheese, therefore, a "starter" composed of a pure culture of Bacillus acidi lactici is added to the milk in order that it may be present in abundance in the ripening cheese. This microbe can not only convert milk sugar into lactic acid, but can also dissolve the casein of milk, changing it into soluble substances, provided that the acidity (produced by lactic acid) be removed by the addition of a neutralising substance like chalk. Another fact that has come to light is that green mould (Penicillium glaucum) which so often attacks bread, preserves, etc., is one of the chief agents in the ripening of Roquefort cheese, in the preparation of which green mould is scraped off bread and added to the curds. In the same way the flavour and colour of Gorgonzola are also largely due to species of Penicillium, whilst other moulds are encouraged to grow upon the surfaces of soft cheeses such as Brie cheese. It is therefore obvious that each kind of cheese will have ripening organisms peculiar to it. In most cheeses the discovery of the ripening organisms is a difficult matter, as can be seen by the fact that one investigator has obtained 80 different species in the samples examined by him. All attempts to ripen cheese by means of any one organism have hitherto been unsuccessful, though good results have lately been obtained by using a combination of organisms.

Defects of Cheese. As so many bacteria can gain access to the milk, to the curds, and to the crude cheese, it is not surprising that serious defects are by no means uncommon in the ripening of cheese. A common defect is the formation of *large holes throughout the cheese*, making it porous, shapeless, and worthless. This is due to excessive multiplication of one of the gas-producing bacteria. At least twenty-five species are known that have this power of causing abnormal swelling in cheese. It does not follow, however, that their presence is always harmful—harm arises only where they multiply extensively. The abnormal swelling may be due to the absence of cleanliness in handling the milk, or to the use of milk from diseased udders. The presence of acid and salt appear to inhibit the activity of these bacteria, and of course the employment of a starter still further handicaps them.

Bitter Cheese is an abnormality which is due to the predominance of Bitter-soft-cheese-bacillus (Tyrothrix geniculatus), or Bitter cheese-coccus (Micrococcus casei amari), or Weigmann's bitter-milk-bacillus, or Conn's bitter-milk-micrococcus, or some other microbe with similar properties. They all belong to the casein-digesting bacteria: they fortunately do not under normal circumstances obtain a chance of multiplying extensively.

Colour in Cheese. Red Cheese may be due to bacteria or to yeasts. Two species of bacteria belonging to the micrococcus group, and producing red decomposition-products when fed on cheese, have been isolated. Also the yeast, Saccharomyces ruber, is known to affect cheese in the same way. A thorough disinfection of the cow stalls whence the milk has been derived was found to be a sufficient guarantee against the ravages of the yeast, and there can be little doubt that general cleanliness will also prevent the bacterial pests from multiplying extensively in the cheese.

Black Cheese may be due to the presence of iron in the milk, as, if the milk be slightly sour and rusty buckets be used, some of the iron will be dissolved, and if, as often happens, traces of sulphuretted hydrogen are present, then the black sulphide of iron will be produced and impart its colour to the cheese. Certain moulds and yeasts, however, by feeding on the cheese and giving rise to black decomposition-products, may be responsible for this colouring.

Blue Cheese is due to the activity of a bacillus, but very little is known about it.

Putrid Cheese is undoubtedly due to the capture of the cheese by saprophytic bacteria and other microorganisms. When one remembers that the byre contains many millions of these bacteria, derived from excrement and other filthy matters, it is a simple deduction to affirm that want of cleanliness is the cause of this malady in cheese.

Poisonous Cheese is a more serious matter. In 1883 and 1884 the intrusion of a disease-germ into cheese in Michigan, U.S.A., caused an outbreak of cheese-poisoning affecting in all about 300 persons. This microbe gave rise in the cheese to a poisonous decomposition-product to which the name *tyro-toxicon* was given. In 1901 a similar outbreak occurred in London. This was caused by a Dutch cheese, and about 17 persons were affected. In the latter case no deaths were reported, and the symptoms disappeared after 48 hours.

§ 4. TANNING.

The process by which hides are converted into leather is known as **tanning**. The operation may be divided into three stages:

I. The unhairing of the hide.
II. The expansion of the hide.
III. The introduction of the tannin.

I. With regard to the unhairing, not only must the hairs be removed, but also the two superficial layers of the hide, known respectively as the epidermis and the mucous membrane. Of several methods in use, the best known are the **sweating** and the **liming** or **slackening** processes. When hides are kept damp putrefaction sets in, and the sweating process consists in placing the hides at a moderate temperature in a chamber saturated with moisture. They are allowed to putrefy just sufficient to enable the hairs and the two superficial layers to be easily scraped off with a knife. The putrefaction is entirely accomplished by bacteria, which are always present both on the hides and in the surrounding atmosphere. They attack the hides just as they do all other moist organic substances, which are left exposed to their action. In the **liming** process, the hides are soaked in baths of milk of lime, at first in weak, and later in progressively stronger solutions, until finally they become saturated with this substance. This process is of course a purely chemical operation; but it has been found that some bacteria, which are present in the baths, retard the introduction of the milk of lime into the hides, so that even in this process the fermentations set up by bacteria cannot be ignored. Of the bacteria that are present in the sweating process, we know next to nothing; but as we are dealing with a putrefactive process, the causal agents will have to be sought for among the saprophytic bacteria.

II. The second stage is the preparation of the hide for the introduction of the tannin. In the case of those hides that have been unhaired by the liming process, the lime with which they are saturated must be removed. This is done by soaking the hides in what is known as a "pickle" or "bate," which consists of a mixture of barley, husks, bran, excrement of dogs, fowls, etc., in which fermentation has already set in. Lactic acid is developed as a result of this fermentation, and this it is which, acting on the insoluble lime, changes it into calcium lactate. Now this latter substance is soluble in water, and can therefore be easily removed from the hides. During this process, however, a change

of a more important kind takes place, viz. a swelling of the hides to almost double their former thickness. The swelling must also probably be attributed to the lactic-acid bacteria, for some of them produce gas copiously as well as lactic acid, and it is the expansion of this gas which causes the swelling.

With regard to the hides that have been unhaired by the sweating process, these are also placed in the bate, not because lactic acid is necessary in their case, but because of the swelling that takes place as a result of the evolution of gas.

It seems strange that in such an important industry these crude methods should still be adopted to effect the swelling of the hides. The introduction of lactic acid must be effected by fermentation, and not by purely chemical means, because apparently the latter method does not turn out a durable form of leather. Now bran and faecal matter, when undergoing putrefaction, contain many varieties of bacteria, and the industry would gain considerably if the manufacturers could ascertain which kinds liberate lactic acid and produce gas. If this were done, they would be able to dispense with the objectionable material with which they work, and by using pure cultures would very probably be able to expedite the operation of liberating the lime and swelling the hide. One step has already been made in this direction by the isolation of one of these bacteria, to which the name **Bacterium furfuris** has been given. This was isolated from a sample of bran in which putrefaction had already partially set in. There is a large quantity of starch in bran, and likewise a ferment called **cerealin**, which changes this starch into a kind of sugar. **Bacterium furfuris** was found to thrive on this sugar, changing it into organic acids and at the same time causing the liberation of large quantities of the gases, carbon dioxide, oxygen, nitrogen, and hydrogen. The individuals of this species are described as consisting of short rods, 0·7 μ long and 1·3 μ broad: they are often united to form chains, and do not form spores.

III. After the hides have been sufficiently "plumped," they are ready for the final phase, viz. the introduction of tannin. In the **bark-tanning** method, the tannin is derived from the bark of various trees, from gall-nuts, myrobalans (dried fruit from East Indies), sumach (dried leaves containing much tannin), etc. The hides intended for sole leather are placed in the tan-pit in such a way that each hide has above and below it a layer of broken bark, powdered gall-nuts, etc., and at the same time *a portion of spent tan from the previous tanning*. After filling the pit with water, the hides are allowed to remain in it

for from eight to ten weeks, after which they are transferred to another pit, where they remain for another period of from eight to ten weeks. This process is repeated from three to five times till the hides are impregnated with tan. The thinner hides not intended for sole leather are treated in essentially the same way, except that they are placed in what is called "bark-liquor," an extract of tanning substances, instead of being placed in the tan-pit.

The changes which take place at this phase are not essentially of a fermentative nature, for the actual tanning is purely a chemico-physical operation. It is, however, important to note that the quality of the leather is controlled by the microorganisms that are found in the tan-pit or bark-liquor. As the tanning proceeds the liquor gets sour, owing to the development in it of an acid, through the agency of these microorganisms. Now the tanner watches the development of this souring very narrowly, because if absent or improperly regulated the leather becomes inferior in quality, and the reason for the introduction of spent tan from the previous tanning into the tan-pit lies in this, that he knows by experience that this spent tan facilitates the souring. The spent tan, in fact, contains multitudes of lactic acid bacteria, and it is the multiplication of these, and the consequent formation of lactic acid, that causes the sourness of the tan-pit. One of these has been isolated. It is called **Bacillus corticalis**, and was found in bark-liquor. The individuals are short rods—0·7–1·0 μ broad and 1·5–2·0 μ long. The species thrives best at 30°–40° C. and stops multiplying below 5° C. It ferments sugars (*e.g.* dextrose, saccharose, and lactose), producing lactic acid and a considerable quantity of gas: it does not act on tannin itself. This species cannot be the only one causing sourness in the tan-pit, but the amount of investigation on this subject has hitherto been very meagre. It must also be borne in mind that there probably are other species in the tan-pit, with properties inimical to the tanner, in which case it would be highly desirable if the tanner could work with starters and regulate the souring of the tan-pit in the same way as the modern butter maker regulates the souring of milk.

§ 5. RETTING.

This is the term applied to the steeping of flax, hemp, etc., for the purpose of loosening the fibre from the other portions of the plant, by the softening of the non-essential parts. The fibre alone by this process escapes the softening, and can therefore be easily separated.

Retting of Flax. Flax is prepared from certain fibres of the plant Linaria vulgaris. These fibres are bound together by the middle lamella (Fig. 128), which is the name given to the material with which the fibres are bound together, and which differs in constitution from the fibres themselves. The object of retting is to effect the solution of the middle lamella, without injuring the fibres. This is accomplished either by **dew-retting** or by **water-retting**. In the former method the plants are spread on the ground and exposed to the influence of the atmosphere, whilst in the latter method the plants, wrapped up in bundles, are steeped, roots downwards, in water-tanks. In both cases, the solution of the middle lamella is accomplished through the activity of certain bacteria, which feed on this binding substance without injuring the fibres. One of these bacteria has been isolated by Winogradsky. This is a bacillus with individuals 10–15 μ long and 1 μ broad, which forms spores, becoming at the same time tadpole-shaped. It grows anaerobically and in the presence of nitrogenous food is able to ferment saccharose, lactose, and starch. A patent has been obtained in the United States for a method whereby the retting can be performed in a few days. This method consists in steeping the plants, after cleaning, in a liquid containing substances which favour the growth of this bacillus, the latter in the form of a pure culture being added to the liquid. It is claimed that this method shortens the retting to about a quarter of the time required in the other methods. There can be no doubt that this is a step in the right direction, and it is surprising that so little attention has been paid to the possibilities presented by pure culture methods of treatment.

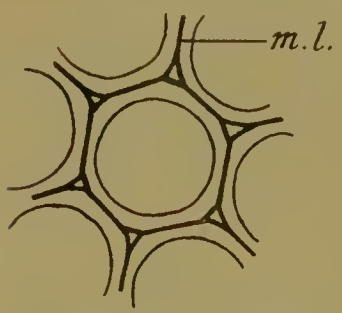


FIG. 128.—Diagrammatic representation of fibrous-cells. *m.l.* = middle lamella.

Retting of Hemp, Jute, and Cocoanut Fibre. **Hemp** is obtained from the fibres of various plants, *e.g.* Datisca, Cannabina and Musa texilis. **Jute** is a fibrous substance resembling hemp but is obtained from the bark of Indian plants allied to the lime-tree. It is largely used in the manufacture of carpets, bagging, curtains, etc. Both hemp and jute are prepared by water-retting, the fibres or the bark being left exposed in rivers or tanks. The fibre used in the manufacture of cocoanut matting is prepared from the outer husk of the cocoanut—the article sold in the shops is the inner part only of the whole nut—and its preparation also is accomplished by the same method. A large field of work is

open in these branches of the industry, but as yet we are not in a position to say more than that the whole process is controlled by bacteria.

§ 6. TOBACCO.

This industry begins to have greater interest for us at the present day, because of its growing importance in Ireland, and because of the growing recognition of the fact that the plant can be easily cultivated in this country. In view of the recent withdrawal of restrictions on its growth in Scotland, we may confidently look forward to a still wider expansion of the tobacco industry.

Tobacco is obtained from plants which are natives of tropical America and Eastern Asia. It is a herbaceous plant, three to six feet high, with large, somewhat hairy leaves. There are two main phases in the preparation of this leaf.

1. The curing process;
2. The sweating or fermentation process.

1. **Curing.** The leaves are hung up in barns, suspended in rows from long laths, in order to undergo a partial drying. They consequently wilt and take on a somewhat brown colour. It may take four weeks or more before the right condition has been reached. During this time the moisture, temperature, and ventilation are judiciously regulated, for the acquirement of a good flavour depends on the proper regulation of these conditions as well as immunity from the attacks of moulds and other pests.

The development of the flavour has been carefully studied but not explained. At first there is a decided flavour of cucumbers; this is replaced later on by the straw smell of cured tobacco. The further development is completed during the second stage. The curing effects a change of colour, the leaves assuming the familar brown colour of wilted leaves. The chemical composition is naturally altered by the curing, the most important changes are: (1) a change of the starch into sugar; (2) a partial disappearance of the sugar; (3) a decomposition of proteid matter, with formation of amido-compounds; (4) a decrease of fatty matter and of tannin. In fact these changes are the same as those which most leaves undergo during the process of wilting, but what is still unexplained is the development of the substances in the tobacco-leaves which cause them to be different from all other leaves.

2. **The Sweating or Fermentation Process.** Fermentation produces further changes in the substances formed during the curing. This process may be effected in either of two ways. According to one method, the leaves are left hanging a long time, being then packed closely in boxes in a very slightly damped condition. They are then left to themselves for several months. Fermentation develops without any further care being bestowed on them by the grower. The boxes are finally opened, when the leaves will be ready for the manufacturer. The other method, which produces better tobacco, is fermentation in open piles. It can be employed in temperate climates only if artificial heat be used. A number of leaves are tied together at the base, forming what is technically called a "hand." These "hands" are well shaken to admit air to every part, moistened if necessary, and then heaped together to form a large pile, each about four to five feet wide and twelve to fifteen feet long. Each is made so that the butts point to the outside. The surrounding atmosphere is kept warm and steam passes freely into the air to secure uniform moisture. All this results in a rise of the temperature of the leaves which, in three or four days, reaches 52° C. (126° F.), or higher. Repacking becomes now necessary to check the rise of temperature. A new pile is made, but this time, to secure uniformity of treatment, the lower "hands" are placed on the top and the outer ones in the centre. The temperature will again rise, but this time more slowly, so that the next repacking will not be necessary for about seven or eight days. Altogether the piles are repacked some five to eight times. This process naturally effects wide changes in the constitution of the leaves. There is a decrease in the quantity of nicotine, tannin, and potassium nitrate, whilst the sugar disappears altogether. These substances are oxidised, giving rise to a number of other substances. The result of all these changes is to cause the leaf to assume a darkish brown colour, when it is ready for the manufacturer.

Nature of this Fermentation. Diverse opinions exist with regard to the nature of the changes that have taken place. Three points have to be noted:

1. The large rise in temperature when the leaves are packed.
2. The oxidation of the nicotine, tannin, etc.
3. The development of the aroma and flavour.

According to one view the oxygen of the atmosphere is held to be sufficient to explain the oxidations, whilst bacterial action is held responsible for the development of heat and for the production of the aroma and flavour. Another view makes bacteria responsible for all

three changes. In fact, Suchsland, the originator of this view, isolated some of these bacteria from Havana tobacco, and introduced them into the fermenting leaves of German tobacco. No practical results have, however, been yet achieved by this mode of procedure. Other investigators, following these lines, have described the various bacteria concerned in fermentation. These are Bacillus mycoides and Bacillus subtilis, two very common species, as well as five new kinds, named respectively Bacillus tobacci I., II., III., IV., V. One or more of these are supposed to be present among, and to be responsible for all the changes that take place in the fermenting leaves.

A third view, which finds most favour, is that certain chemical substances are present in the leaf, which are capable of exercising a very strong oxidising action. All the changes are claimed to be due to the oxidations of these substances. They are also claimed to be **oxidising enzymes.** Loew's experiments prove beyond a doubt that such oxidising agents are present in the leaf, and that the great heat developed by fermenting leaves is mainly due to them. His experiments do not, however, prove that they produce all the changes, and it is still a matter of doubt whether the aroma and flavour, which are the most important factors, are due to them. If we assume the presence of bacteria, it becomes easier to explain why it is that different kinds of tobacco have such different flavours, for the bacteria found on the tobacco leaves of different countries would naturally not belong to the same species. The practice of **petuning** lends colour to this supposition. By this process a liquid is sprayed on the fermenting leaves either during or after the fermentation process. The petuning liquid is supposed to give the peculiar flavour to Cuban tobacco. Its composition is kept a secret. The changes described by Loew take place in all fermenting tobacco leaves. All that can be said at present is that the rôle of bacteria in the fermenting of tobacco has not yet been determined.

§ 7. INDIGO.

This dye is obtained from a number of leguminous plants, native to the East and West Indies, the best known being **Indigofera tinctoria.** The plants are placed in cisterns, and kept at 25°–35° C. for 8–15 hours. The glucoside **indican,** which is contained in them is transformed, through the activity of a special bacillus which is found on the leaves, into **indigo-white,** and a sugar called **indigo-glucin.** On the surface of the water, however, the colour, instead of being of a greenish yellow

hue, is blue. By violently stirring the whole mass, and thus introducing oxygen, the liquid becomes blue throughout. The colouring matter which gives its hue to the whole mass is called **indigo blue.** That the production of this dye is due to bacteria, is shown by the fact that its formation does not take place if the leaves are sterilised. The microbe in question has been isolated. It is a short bacillus, which is enveloped by a clearly visible mucilage layer.

§ 8. TEA AND COCOA.

Tea is prepared from the young leaves of **Thea chinensis,** which grows wild in Assam, and possibly also in China, but is now cultivated in many parts of the globe. So far as the preparation of green teas is concerned, fermentation does not come into play, because the leaves are roasted immediately after being cut. In the preparation of black teas, however, the cut leaves are exposed to the sun, in order to enable a fermentation to be set up. The changes that take place during fermentation are very little understood, but one change is the conversion of a good part of the tannin that is in the leaves into tannic acid and sugar. The planter knows by experience the exact moment to stop the fermentation. The leaves are then removed to ovens, and roasted. It is probable that oxidising enzymes are present, and that they are largely concerned in the preparation of the leaves. It is unknown whether bacteria come into play in the formation of the alkaloid **theine,** the presence of which in tea forms the sole excuse for its consumption.

Cocoa is prepared from the seeds of **Theobroma cacoa.** The seeds are mixed with plantain leaves and placed in barrels, the latter being then hermetically sealed. Anaerobic fermentation sets in, which is allowed to proceed for from four to seven days. The seeds are then taken out, spread on trays, covered with red earth, and left for another day to complete the fermentation. It is probable that the organisms responsible for the fermentation are of a bacterial nature, and that these are normally present in or on the plantain leaves. We have no information as to the changes that take place during this fermentation.

CHAPTER XVIII.

SEWAGE AND SEWAGE DISPOSAL.

§ 1. INTRODUCTION.

For the purpose of this chapter, sewage may be described as a liquid which holds in solution certain organic matter obtained both from animals and plants; containing also animal excreta, vegetable remains, and solid débris of animals and plants. Further, as decomposition takes place very rapidly, numerous other bodies will be formed as products of this decomposition. There will also be inorganic matter in suspension, such as grit, gravel, street-washings, etc., and various elements in solution, such as phosphates. The whole constitutes a medium which is exceptionally favourable to the growth of certain kinds of bacteria. Unfortunately, as the numerous epidemics arising from sewage-contaminated drinking water have shown, many disease-bacteria lurk in sewage, so that its disposal is a matter of prime importance to the community. The immense number of bacteria which sewage can support is shown by the following table:

Sample of Crude Sewage.	No. of bacteria per cub. cm.
1. Chiefly Domestic Sewage,	14,200,000
2. Mixed Sewage,	7,800,000
3. Chiefly Domestic Sewage,	4,800,000
4. Mixed Sewage and Trade Effluent,	36,000,000
5. Hospital Sewage,	2,800,000
6. Domestic Sewage and Trade Effluent,	4,100,000
7. Domestic Sewage,	28,100,000
8. Mixed Sewage,	21,100,000

These bacteria are engaged in the work of breaking down the complex organic products. Speaking broadly, they effect the same changes as

the bacteria which feed on manure. Ammonium compounds are largely produced, these in their turn being successively changed into nitrites and nitrates. The latter again may be partially still further reduced by denitrifying bacteria and free nitrogen liberated. As there are so many different kinds of bacteria in sewage, a very large number of diverse substances are produced as a result of their activity. Many of these are gases, so we find carbon-dioxide, hydrogen, sulphuretted hydrogen, and marsh-gas in sewage. If sulphur-bacteria are present, the sulphuretted hydrogen will be converted into sulphates. We know as yet very little of the actual processes that take place during the decomposition of sewage, or of the precise rôle played by the various bacteria, we only know the general trend of the decompositions. The work of the majority of these bacteria is of a highly beneficent nature in sewage as it is in manure, and in all places where organic matter is being broken down: the saprophytic and nitrifying bacteria, by using up the organic matter, changing this into other substances, prevent the multiplication of the pathogenic bacteria which are also always present in sewage. If unchecked, the latter would also consume the organic matter in sewage, but would change it into, amongst others, a number of highly poisonous bodies.

A list of the principal sewage bacteria has been furnished by Dr. Rideal, which is as follows:

(L. = liquefying gelatine. S.L. = slightly liquefying. N.L. = not liquefying.)

Obligatory anaerobes.

Spirillum rugula (very active: gives rise to faecal odour).
Sp. amyliferum.
Bac. enteritidis sporogenes.
Bac. amylobacter, L.
B. butyricus, L. (gives much gas).

Facultative anaerobes or aerobes.

B. putrificus coli, N.L. (decomposes albuminous substances, with liberation of ammonia, whether air is present or not).
Spirillum plicatile, serpens, undula, tenue, and volutans.
Vibrio saprophilus, aureus, flavus, flavescens, N.L.
B. mycoides, L. } produce ammonia from nitrogenous organic
Proteus vulgaris, L. } matter and denitrify.
B. fluorescens liquefaciens, L.; and non-liquefaciens, N.L.
Micrococcus ureae, N.L. Bac. ureae, N.L. (convert urea into ammonium carbonate).
B. mesentericus, L.
Proteus mirabilis and P. Zenkeri, L.

B. megatherium, L.; liquefaciens, L.; magnus; spinosus.
Streptococcus liquefaciens coli, L.; and mirabilis, N.L.
B. saprogenes I., II., and III.; pyogenes and coprogenes fetidus.
B. acidi paralactici.
B. lactis aerogenes, N.L. (produces CO_2 and H).
B. coli communis, N.L. (produces much gas, mainly H).
Cladothrix dichotoma, L.
Proteus sulphureus, L (produces H_2S and mercaptan).
Bacterium sulphureum, L. (produces H_2S).
Beggiatoa alba (oxidises H_2S into sulphuric acid).

The following reduce nitrates to nitrites.

B. vermicularis, liquidus, ramosus, aquatilis (also B. myeoides and Proteus vulgaris).

This list does not include all the bacteria found in sewage, but only those more commonly found there. So many different kinds of waste matters find their way into sewage, that a complete list would probably number several hundred species of bacteria.

We will now deal with those species that have a special interest for us, owing either to their pathogenicity or to their abundance in sewage. It is well to have a more intimate knowledge of the latter kind, because it is by their means that sewage contamination can best be detected. The most dreaded of the pathogenic bacteria in sewage is Bacillus typhosus, which gives rise to typhoid or enteric fever. But B. typhosus does not multiply rapidly in sewage; for even in sterilised sewage, in which there is naturally no competition with other organisms, it fails to grow, and indeed quickly perishes. It seems to lead a very precarious existence in sewage, and cannot strictly be called one of the sewage bacteria. It often, however, finds its way into sewage; and as drinking water sometimes becomes contaminated with sewage, we see how it is that this dreaded microbe enters the human system. The cause of the fever being known, it is sad to reflect on the enormous waste of life that took place during the late South African War through the ravages of this disease. The number of soldiers who died of enteric fever considerably outnumbered those who died of wounds. The characteristics of Bac. typhosus and its effects on the human system have already been described. There is great similarity between Bac. typhosus and Bacillus coli communis, an organism which is very common in sewage, and which is strongly suspected of being the cause of epidemic diarrhœa, though positive proof is still wanting. It is found as a normal inhabitant of the alimentary canal of most mammalia, is widely distributed, and thrives

well in sewage. On the average there are 100,000 individuals of this species in one c.c. of sewage. The individuals are rod-shaped, short, and with well-rounded ends. They measure 2 μ to 3 μ in length and 0·5 μ to 0·6 μ in breadth, and are normally motile. The prevalence of this species in sewage, and the ease with which it can be isolated, are often taken advantage of, when it is desired to prove that a certain water had become contaminated with sewage.

Although Bac. coli communis closely resembles Bac. typhosus in general characteristics, recent research has now rendered it easy to distinguish these organisms. The best points of distinction can be tabulated as follows:

	Bac. Typhosus.	Bac. Coli.
Morphology.	Rods unequal length.	Rods shorter and thicker.
Growth on gelatine and agar plates.	Slow growth; colonies slightly raised.	Rapid growth; colonies well raised and larger.
Gelatine culture.	No gas.	Abundant gas.
Milk culture.	Not curdled; no acid reaction.	Curdled; acid reaction.
Bouillon culture.	Indol not present.	Indol present.
Bouillon containing lactose.	No gas.	Gas.
Neutral red-glucose agar.	No change.	Green fluorescence.
Potato culture.	"Invisible" growth if potato has acid reaction.	Thick yellow-white growth.

In addition to these, there are other tests, dependent on differences of growth in various media, *e.g.* Elsner's iodised potato gelatine, M'Conkey's lactose agar, and Proskauer & Capaldi's medium. It must be remembered that Bac. coli communis is the name rather of a group of closely-allied organisms than of one species, and the above tabulated characteristics refer to what may be called the central species. There are at least sixteen varieties, all of which agree in being non-sporing and non-liquefying; in coagulating and producing acid in milk; in producing acid and gas in glucose and lactose media; in producing acid and gas in bile-salt-glucose broth; in growing well at 42° C. Further, there are other closely-allied organisms which differ from this central species only in some points. These have been collected into five groups:

Group A. Ferments lactose; coag. milk; no indol reaction.
„ B. „ not coag. milk; gives indol reaction.
„ C. „ „ no indol reaction.
„ D. Do not ferment lactose; coag. milk; no indol reaction.
„ E. „ not coag. milk; no indol reaction.

Another organism which is potentially pathogenic, and very common in sewage, is **Bac. enteritidis sporogenes.** This is credited with being the cause of the autumnal diarrhoea of children and the "English cholera" of adults. It is widely distributed, and occurs in normal and typhoid excreta, so it is always well represented in sewage; it is anaerobic in its mode of life, and the rods measure 1·6–4·8 μ in length and 0·8 μ in breadth. Further, the individuals are motile and normally form spores, which are large, oval, and situated near one of the ends of the cell (Fig. 129). The growth of the species in gelatine and in agar is attended by gas production, and in the former liquefaction also takes place. It likewise grows well in milk, producing what is known as the "enteritidis change": after 36 hours of anaerobic incubation in milk at 37° C., the cream is dissociated by the development of gas, so that the surface of the medium is covered with stringy pinkish-white masses of coagulated casein, enclosing a number of gas bubbles. On the average there are about 100 spores of Bac. enteritidis sporogenes in one c.c. of normal sewage, but being in the spore condition the species can be easily isolated by boiling the sewage sample for a couple of minutes before making the usual plate cultures: the boiling kills all the bacteria that are not in the spore condition, thus greatly increasing the chances of the appearance of this species on the plates. The ease with which this species can be isolated, its well-marked morphological characteristics, especially in the sporogenous condition, and its abundance in sewage, have all combined to make this organism valuable to the sewage expert, as one of his chief bacteriological means of detecting sewage contamination.

Fig. 129.—Bac. enteritidis sporogenes.

Another class of closely-allied organisms found normally in sewage, sometimes in very large numbers, is the Proteus group, mention of which has already been made in dealing with the work of the saprophytic bacteria. The group includes Proteus vulgaris, P. mirabilis, P. Zenkeri, and P. cloacinus. They must be treated here in greater detail, because they thrive well in sewage, and there may be as many as 1,000,000 individuals of one or more members of the group in one c.c. of sewage. As a class the members may be distinguished by the following characteristics: They are motile; they liquefy gelatine; they produce gas in glucose and sucrose, but not in lactose media; they curdle and acidulate milk, but very slowly; they produce indol; they do not form endospores; they are strongly aerobic; and, when grown in albuminous media, cause the formation of foul-smelling products.

Proteus vulgaris is the best known. The rods are 0·9–1·2 μ in length, 0·4–0·6 μ in breadth, and almost always occur in couples. In addition, elongated forms occur frequently, attaining a length of 3·7 μ and more. In Fig. 130 a ciliated long and a similar short rod are shown. As is evident from the large number of cilia possessed by this species, the motility is very pronounced. Indeed, motility is a characteristic possessed by all the members of this group. As already

Fig. 130.—Proteus vulgaris. Showing a ciliated long and ciliated short rod.

mentioned, the term "Proteus" refers to the variety of the forms assumed by the individuals of the same species; and in Proteus vulgaris not only do we have the long and short individuals, but also spirilla with two to four convolutions, threads which are 100 μ and more in length, threads bent in the form of a bow, with ends twisted into a queue. These are normal structures. When involution forms are exhibited, we see more than the usual variety in shape; thus, there are pear-shaped and dumb-bell shaped individuals, amongst many other departures from the rod form.

Proteus mirabilis also shows many departures in the shape of its individuals, a common form being globular or pear-shaped structures measuring from 3 μ to 7 μ across. This species is very similar to P. vulgaris in most respects, but one distinctive feature is that in this species some threads may attain a length of 200 μ, *i.e.* double the maximum size of the threads of P. vulgaris.

Proteus Zenkeri differs from the two preceding species in being unable to liquefy gelatine and having normally smaller and shorter rods (about 0·8 μ long). In addition, there are numerous small globular individuals, about 0·4 μ in diameter.

Another group which needs a short description is the one of which **Bac. lactis aerogenes** forms the central type. The members of this group are non-motile, do not liquefy gelatine, but are able to curdle and acidulate milk, and can ferment sugars other than glucose.

The rods of Bac. lactis aerogenes are 0·5 μ to 0·9 μ broad, and 1 μ to 2 μ long, are non-motile, and do not form spores. In milk the

rods often form chains, and, as the ends of the rods are sharply rounded, a chain gives the impression of a row of cocci. When grown in bouillon, the latter is rendered turbid, and a characteristic slimy film is formed at the top, whilst at the bottom a slimy sediment is produced which can be drawn into long threads.

Next is a small group clustering round the Bac. enteritidis of Gaertner. This bacillus is in the main not unlike Bac. typhosus, but differs from it in growing more rapidly in gelatine, in possessing fewer cilia, and in being able to ferment lactose, and sometimes dextrose. Like Bac. typhosus, it can produce indol, coagulate milk, and is unable to form endospores.

Finally, it is important to note the Streptococci that are found in sewage, which in the crude state contains on the average about 1000 of these organisms per cub. cm. Their importance to the sewage bacteriologist will be shown in the next paragraph.

§ 2. DETECTION OF SEWAGE CONTAMINATION.

It is important to be able to identify the commoner of the sewage bacteria, because the power to do so furnishes us with an extremely delicate method of finding out whether drinking or other water has been contaminated with sewage or faecal matter. The chief organisms for which search is made are Bac. coli communis, Bac. enteritidis sporogenes and Streptococci. These are as stated above very numerous in crude sewage, are absent from pure water, at least in any considerable numbers, and are easy to identify. Houston's "standard of crude sewage" states that one cub. cm. contains

1. 1–10 million bacteria.
2. 100,000 Bac. coli communis or allied forms.
3. 100 spores of Bac. enteritidis sporogenes.
4. 1000 Streptococci.

Bac. coli communis, Bac. enteritidis sporogenes and the Streptococci have consequently been termed the "microbes of identification." Of these three Bac. coli communis gives the most accurate measure of intestinal or of sewage pollution, because it is an intestinal parasite and tends to perish in other media; so if present in fair numbers in drinking water, it shows that there must be food for it, which should not be the case in drinking water. The presence of Streptococci in water is an indication of *recent* and dangerous pollution. These organisms are entirely absent, or present only in small quantities in

pure water and in virgin soils. As a class they are delicate germs that soon lose their vitality under unfavourable conditions, and several of the species are pathogenic to man. Hence if found in drinking water in large quantities, they must have got there by pollution of the water with human faeces or with sewage, in both of which they abound; being delicate organisms their presence in large numbers indicates that the pollution is recent.

§ 3. THE TREATMENT OF SEWAGE.

It will be seen from the above statements that it would be extremely imprudent to allow sewage to escape in large quantities without subjecting it to some treatment which would prevent the possibility of the multiplication of the pathogenic germs contained in it. If not so subjected the sewage of a town with a fairly large population might become a serious menace to the community. There are three obvious methods which present themselves for consideration. We may conduct the sewage, without treating it in any way, to a place where it can do no harm, or we may kill off the contained bacteria by heat or by an antiseptic, or finally we may so change the constitution of sewage that it becomes unfit for the pathogenic bacteria to live in. Of these the first is possible where the quantity of sewage is small: the second is not practicable; whilst the third method can be used for large and small quantities alike. It will be seen that in all the methods of artificial treatment, the underlying principle is the removal of the bacteria by the removal of their food supply. In connection with this point, it is interesting to note the manner in which the sewage-bacteria are kept under in nature, when they enter into competition with other organisms. In Chap. VI. we have shown how the sewage-bacteria, which enter the Severn in large numbers as the waters of that river pass through Shrewsbury, diminish in numbers to such an extent that the water a few miles lower down is as pure as it was before it reached Shrewsbury. It was shown that one of the chief agencies concerned in this self-purification of the river is the rapid diminution of the food supply due to the fact that the food contained in sewage is keenly competed for, not only by the sewage-bacteria but by a host of other organisms. Amongst these competitors the most formidable are the other non-pathogenic bacteria, for it is a rule in nature that the more nearly allied the organisms, the keener becomes the struggle for existence; and the

conditions of life for pathogenic bacteria and their bacterial competitors are almost identical. All the biological methods (both land and artificial treatment) have this end in view, viz. the consumption of the food supply by non-pathogenic bacteria.

We may distinguish three main methods of sewage disposal:

1. Disposal without purification.
2. Land treatment.
3. Artificial treatment.

§ 4. DISPOSAL WITHOUT PURIFICATION.

In small villages the sewage is not usually collected together, each house disposing of its own portion as best it can. The faecal matter is usually covered with earth and periodically removed, whilst in the better-class houses the same material is drained off, the pipes discharging their contents into cess-pools. The liquid part gradually wells up to the surface and as it passes through the earth its organic content becomes gradually converted by the soil-bacteria, into substances that cannot serve as bacterial food. The solid portion remains in the soil, where it is out of harm's way and where it is speedily disintegrated and chemically changed by the activity of anaerobic bacteria. The other substances that in larger places usually find their way to the sewage-drain are thrown broadcast on to any convenient spot, such as a roadside or a neighbouring common. This method is efficient enough for very small places, though it must detract somewhat from the healthiness of village life. In most small towns situated on or near the banks of a river or on the sea-shore, the sewage is conducted into the river or sea as the case may be, with, in the majority of cases, harmless results. We have explained above, the manner in which purification is effected by rivers. The method is attended with danger to the inhabitants of the houses situated near the outlet of the sewage into the river, and may injuriously affect their health. The area of danger extends to at least four miles below the outlet of the sewage. In the case of sewage conducted to the sea, there is danger if bathing grounds are located in the immediate neighbourhood, and there are cases known of epidemics of typhoid fever, caused by the consumption of shell fish gathered from places near to the outlet of sewage into the sea.

§ 5. LAND TREATMENT OF SEWAGE.

This is done by one of two methods, known respectively as **Intermittent Filtration**[1] and **Broad Irrigation.**[1]

By the first method the sewage is placed on the land and allowed to filter through the soil, the effluent being drained off by soil pipes (Fig 131). Before reaching the soil pipes, the sewage has therefore to pass through a certain depth of soil. The passage is marked by far-reaching changes in the sewage, which, as will be explained below, becomes radically altered in constitution. The

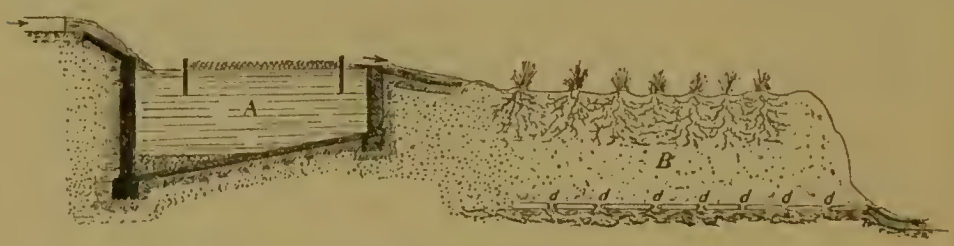


FIG. 131.—To illustrate land-treatment by intermittent filtration. Sewage after treatment in septic tank (A) is allowed to filter through soil (B) and is then collected by soil-pipes (*dd*..). (After Lafar.)

filtration is made intermittent, because a break is necessary to secure adequate aeration of the soil. By the second method the sewage is run over a large area of land, the idea being to spread the sewage over the *surface* of the soil as a thin layer, so thin in fact that the work of purification takes place mainly on the surface. The sewage is not drained off and the vegetation is not affected, in fact the chief endeavour is to secure a maximum growth of vegetation consistent with purification. As in the other method, the laying of the sewage must be intermittent in order to secure proper aeration of the soil.

One or other of these two methods is usually employed in places where plenty of land is available, about one acre to every 100 of the population being required. Every kind of land can be utilised for this purpose, with the exception of peat and stiff clay lands. These are so wanting in porosity, that a very large tract of land would be required for the purpose, so large in fact that land-treatment in clay

[1] The two names *intermittent filtration* and *broad irrigation* have not been happily chosen and have lately been replaced by the self-explanatory terms *downward filtration* and *surface irrigation* respectively. In the text we shall use the older terms because of their familiarity.

and peat districts is impracticable. This is emphasised by the Royal Commission on Sewage Disposal (1898), in their Interim Report.

> "We doubt if any land is entirely useless, but in the case of stiff clay and peat lands, the power to purify the sewage seems to depend on the depth of the top soil . . . We are however forced to conclude that peat and stiff clay lands are generally unsuitable for the purification of sewage, that their use for this purpose is always attended with difficulty, and that where the depth of top soil is very small, say six inches or less, the area of such lands which would be required for efficient purification would in certain cases be so great as to render land treatment impracticable."

As to the changes that take place in the sewage, we have already explained that these are essentially the same as those which take place when organic manure is placed on the soil. The rich organic matter is eagerly seized upon by organisms that are present in the soil, and by those that are already present in the sewage.

The ultimate result will be the production of a number of relatively simple substances which will be quite unfit for pathogenic bacteria to thrive upon. Owing to the hardiness of the soil-bacteria, and owing to the conditions under which the decomposition is taking place, the pathogenic bacteria do not get a chance to multiply inordinately. That partial multiplication takes place is unavoidable, and effluents after land treatment cannot be regarded as absolutely harmless. The justification of land treatment lies in the fact that after treatment the number of pathogenic or potentially pathogenic bacteria is very small, and in addition, the nutriment of these bacteria has disappeared, so that subsequent multiplication will not be possible, at any rate multiplication for which the sewage is responsible.

In the broad irrigation method aerobic bacteria are most active, and of these the nitrite- and nitrate-bacteria play perhaps the most important rôle, so that in place of the organic matter, a number of harmless and useful inorganic products are obtained. In the intermittent filtration method anaerobic bacteria will take more prominent parts in the decomposition, because the work is mainly carried on under the ground. These organisms appear to produce unstable substances that are readily attacked, especially by oxygen. We do not know much of the work carried out by the anaerobic bacteria, but it seems probable that one or two molecules of oxygen or hydrogen, nitrogen or carbon, are snatched from a large and complex molecular group, the group being then left in a very unstable condition and in such an altered form that it readily undergoes oxidation when subsequently

access is obtained to the oxygen of the air. The oxidation is effected partly in a purely chemical manner and partly through the agency of aerobic bacteria.

§ 6. ARTIFICIAL METHODS OF SEWAGE DISPOSAL.

Until recently, the above-mentioned methods were the only ones that were known for the disposal of sewage. But in thickly populated districts the large amount of land required for the purpose made land treatment very expensive, with the result that a number of artificial methods gradually sprang up, some of a chemical, others of a biological nature.

With the various chemical methods of disposal we have very little to do in this book, as bacteria play no part in the changes that are effected in the sewage. A score or more of chemical methods are in use, and with one exception all depend upon the precipitation of sewage by means of various chemical substances. A common method is to add 6-12 grains of quicklime to each gallon of sewage, the result being the formation of carbonate of lime. As the precipitate falls it carries with it the solid organic matter contained in sewage. Many other substances, and combinations of substances, are in use as precipitants, *e.g.* lime and ferrous sulphate, a mixture of clay, carbon, blood and salts of alumina, a mixture of the higher oxides and crude sulphate of manganese and other combinations. The defect from which all chemical methods suffer is that precipitation does not affect the organic matter which is in solution. Of the various methods, many authorities consider that the simplest and cheapest, as well as the most efficient method is lime precipitation.

The various biological methods aim at the destruction of sewage on the same lines as is effected by land treatment, that is, by getting rid of the organic matter by causing it to be changed into harmless products through the agency of various non-pathogenic bacteria. The biological methods have this advantage over land treatment that the sewage is under control, and can be changed from one situation to another, so that different kinds of bacteria can have full scope for their activity. This ensures a more complete breaking down of the organic matter. It also differs slightly from land treatment in that a more important part is played by the sewage bacteria. It may seem strange that the sewage bacteria should be allowed to multiply in this way, but it must be borne in mind that the sewage is

under control, and that only a few of the sewage-bacteria are really dangerous. If the organic matter were allowed to be broken up by the sewage bacteria and the sewage then set free without further treatment, there would be serious danger owing to the possibility of the multiplication of those sewage-bacteria that are pathogenic. But before being set free the sewage is brought under conditions which give the pathogenic or potentially-pathogenic bacteria very little chance of multiplying to any great extent, so that if the organic matter be used up without any undue multiplication of the latter organisms, the end for which these processes were devised, has been achieved. To obtain an insight into the working of the various kinds, it is necessary to understand that there are essentially two phases in the work of decomposition, one in which the anaerobic bacteria and one in which the aerobic bacteria have full scope for their activity either successively or simultaneously. For the anaerobic bacteria break down solid organic matter into liquefiable simpler substances which, as explained above, can be readily attacked by aerobic bacteria in the presence of oxygen.

The first of the biological processes in this country was the "cultivation tank" invented by Mr. Scott Moncrieff in 1891. It consists of a chamber filled with large stones (preferably flints), and carried by a grating. The crude sewage is made to rise slowly through the chamber. As it passes upwards, a large portion of the suspended matter is caught on the stones, the object of the latter being to afford a resting place for the anaerobic bacteria which are certain to collect on them. These bacteria attack the solid sewage thus caught, the result being that the solid matter is liquefied. After passing through the stones the sewage matter is brought into contact with highly oxygenated water, and is then passed through a "nitrification channel" where the organic matter is attacked by aerobic bacteria. The cultivation tank is a special contrivance for favouring the multiplication of the anaerobic bacteria, whilst the nitrification channel does the same for the aerobic bacteria.

Next came in point of date, Mr. Cameron's **septic tank**. By a septic tank is meant one in which putrefaction is taking place. It consists essentially of a chamber through which sewage is allowed to flow continuously, the inlets and outlets being preferably submerged, so as not to interfere with the scum which forms on the surface. It differs from the cultivation tank in that no material, *e.g.* stones, is placed inside to furnish surfaces for the liquefying bacteria to cling to; otherwise it is identical in that the work of disruption is effected by the anaerobic bacteria contained in the sewage itself. The efficiency of the septic

tank as an apparatus for the liquefaction of sewage solids has now been universally acknowledged.

The following classification of the various methods of dealing with sewage biologically shows how different workers have grappled with

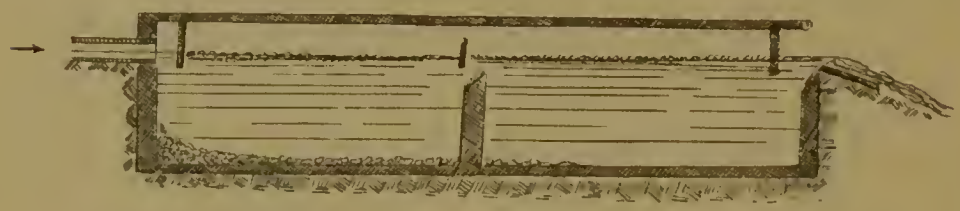


FIG. 132.—Septic tank working under anaerobic conditions. (After Lafar.)

the problem after obtaining a start by the invention of the cultivation- and the septic-tanks. (Figs. 131, 132, 133.)

1. Closed septic tank and contact beds.
2. Open septic tank and contact beds.
3. Chemical treatment, subsidence tanks and contact beds.
4. Subsidence tanks and contact beds.
5. Contact beds alone.
6. Closed septic tank followed by continuous filtration.
7. Open septic tank followed by continuous filtration.
8. Chemical treatment, subsidence tanks and continuous filtration.
9. Subsidence tanks followed by continuous filtration.
10. Continuous filtration alone.

A **contact bed** (Fig. 133 B) is an artificial filter made up of such materials as burnt clay, coke, cinders, gravel, etc. The bed is filled and emptied alternately either with sewage after treatment in the septic tanks (Nos. 1 and 2), or with sewage after chemical treatment (No. 3), or without any preliminary treatment (Nos. 4 and 5). The object of a contact bed is to hold the sewage in contact with the filtering material as long as is necessary to effect oxidation, after which the filter is emptied to allow a fresh supply of air to penetrate its interstices. The action of such a filter is twofold. It separates mechanically all the gross particles of suspended matter, and also effects the oxidation of the organic matter, chiefly through the agency of the aerobic bacteria. To secure the maximum efficiency the organisms must be supplied with plenty of air, the filter must be supplied with a base such as lime to neutralise the nitric acid that will be formed, and finally the filtration must take place in the dark. The **multiple contact beds** first used at Sutton is an example of the fifth method. At Sutton each bed is 3½ feet deep and the filtrant is burnt clay, which is occasionally

forked over to let in the air. The sewage is passed into the bed at the bottom and allowed to rise to within six inches of the top. After remaining in contact with the filter for two hours, the sewage is drawn off, and then passed in the same way to a fine grained contact bed made up of the same material. After this treatment the sewage is considered to be pure enough to be allowed to escape. There is no preliminary treatment by passing the sewage through a septic tank or by chemical

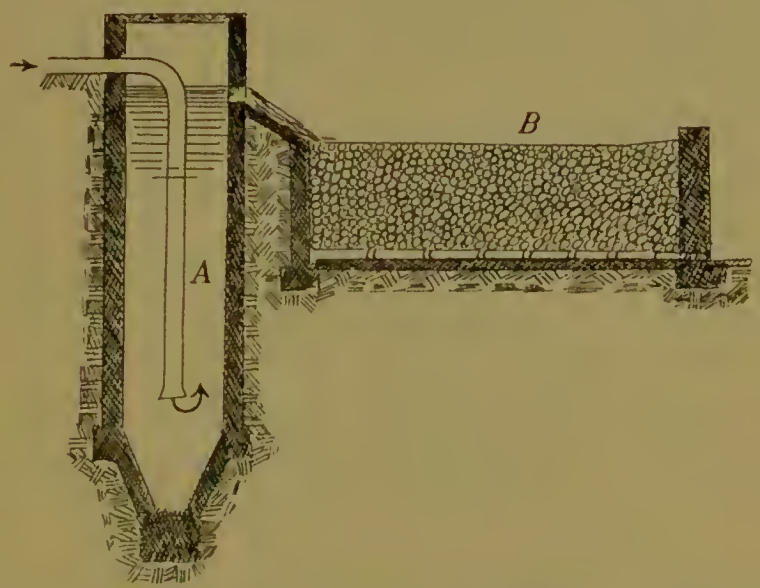


FIG. 133.—To illustrate a common method of sewage-treatment. Septic tank (A) followed by contact bed (B). (After Lafar.)

treatment, because it is assumed that the anaerobic work has been accomplished sufficiently for the purpose in the sewers. Definite experiments have been made to ascertain whether this preliminary treatment can be dispensed with. It was found that it was unquestionably beneficial to the disposal of sewage. In the third method, it will be noticed that the sewage is placed in **subsidence tanks**, after the preliminary chemical treatment and before the subsequent treatment in contact beds. A subsidence tank differs from a septic tank in that little or no "septic" action takes place. They are used to avoid the clogging of the contact beds which would otherwise happen if the precipitate formed during the chemical treatment were allowed to enter the contact beds.

In the remaining methods (Nos. 6-10), whilst the preliminary treatment is the same as in one or other of the first five methods, the method of dealing with the filtration is different. This is accomplished by **continuous filtration**. The liquid is brought into filter

beds, but unlike the method of working the contact beds, it **flows** through the filter without being brought to rest. The filter is consequently not filled and emptied alternately. Continuous filtration may be accomplished either by the **streaming filter** in which the liquid is made to flow out at the same rate as it flows in, or by some form of **trickling filter** in which the liquid is showered or sprayed into the filter instead of flowing in. The majority of trickling filters are fed by what are known as rotary sprinklers (Fig. 134), consisting of pipes

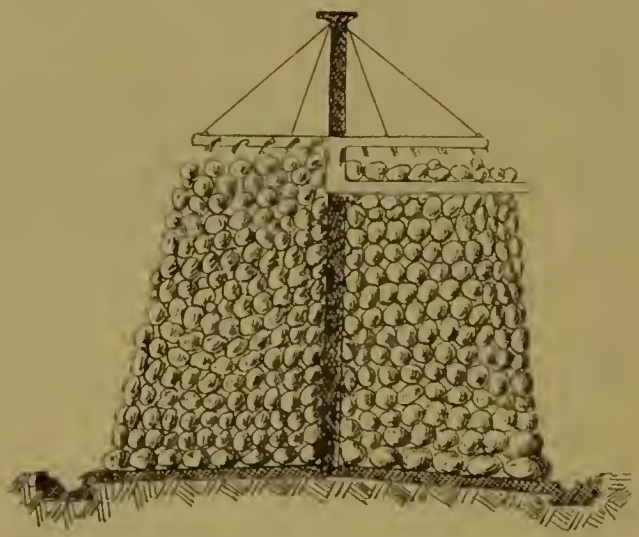


FIG. 134.—Trickling filter with rotary sprinkler. (After Lafar.)

pivoted in the centre of the filter and having perforations on the opposite sides of the two arms, from which the effluent is showered on to the bed, the reaction of the liquid against the side of the pipe being utilised to rotate the sprinkler. As the spindle revolves, the points of impact of the jets are continually changed, thus tracing a series of circles in the filter.

In all these filters adequate oxidation is secured by making the sides or walls as open and porous as possible.

§ 7. EFFECT OF BIOLOGICAL TREATMENT ON PATHOGENIC ORGANISMS.

All the biological methods that we have mentioned are successful in that they are able to alter sewage to such an extent that the effluent contains no putrefiable matter. But the effluent is not free from bacteria, and it is important to know the nature of these bacteria. It

has been shown that some of the bacteria that are present in the effluent are capable under certain conditions of engendering diseases of various kinds, so that it is considered dangerous to allow effluents to enter streams, the waters of which in the immediate neighbourhood are used for drinking purposes. There is at present no known method which can be absolutely relied upon to free sewage altogether from the germs of disease, and we are not yet in possession of much definite information as to the exact extent of the danger arising from the effluents of the existing methods. Houston's work indicates the "inadvisability of relying on septic tanks, contact beds or continuous filters to remove altogether the element of potential danger to health associated with the discharge of effluents from these processes of sewage treatment, into *drinking-water* streams." There can, however, be no doubt that the treatment of sewage is highly beneficial to the community, and we may conclude with Dr. Reid's declaration that if he had to choose between "the discharge into a stream of the crude sewage of 29,000 people" and that "of the treated, therefore non-putrefactive, sewage of a population of 420,000 odd," all he could say would be "it would not be the crude sewage of 29,000 people which he should select in the case of a stream over which he had control, as an alternative to the treated sewage of a population, no matter how large."

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